THE HARVEY SOCIETY

Med.
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THE HARVEY LECTURES

DELIVERED UNDER THE AUSPICES OF

THE HARVEY SOCIETY OF NEW YORK

1916-1917

BY

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SERIES XII

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PREFACE

It was the idea of the founders of the Harvey Society to present to the medical profession the results of laboratory investigation on various phases of problems which would contribute to the advancement of medical science and thereby tend to establish medical practice on a firmer and more rational basis.

The formal entrance of the United States into the European War has led to a demand upon the medical profession as a whole for things hitherto unattempted in prophylaxis and therapy. To meet this demand, the scientific laboratories of the country have been drawn upon to an unprecedented extent in order to furnish the necessary foundation of fact and discovery for the development of the best and most rational medical treatment of the problems of the war. Although written before our formal entry into the conflict, the twelfth volume of the series of Harvey Lectures, and the earlier volumes as well, contain matter of direct interest in connection with the medical problems arising from the war. In so far as the Harvey Lectures contribute in any way to the solution of the problems confronting us in the present emergency, the hope of the founders of the Society will be realized.

F. H. Pike,
Treasurer and Acting Secretary.

March, 1918.

THE HARVEY SOCIETY

A SOCIETY FOR THE DIFFUSION OF KNOWLEDGE OF THE MEDICAL SCIENCES

CONSTITUTION

I.

This Society shall be named the Harvey Society.

II.

The object of this Society shall be the diffusion of scientific knowledge in selected chapters in anatomy, physiology, pathology, bacteriology, pharmacology, and physiological and pathological chemistry, through the medium of public lectures by men who are workers in the subjects presented.

III.

The members of the Society shall constitute three classes: Active, Associate, and Honorary members. Active members shall be laboratory workers in the medical or biological sciences, residing in the City of New York, who have personally contributed to the advancement of these sciences. Associate members shall be meritorious physicians who are in sympathy with the objects of the Society, residing in the City of New York. Members who leave New York to reside elsewhere may retain their membership. Honorary members shall be those who have delivered lectures before the Society and who are neither active nor associate members. Associate and honorary members shall not be eligible to office, nor shall they be entitled to a vote.

Members shall be elected by ballot. They shall be nominated to the Executive Committee and the names of the nominees shall accompany the notice of the meeting at which the vote for their election will be taken.

IV.

The management of the Society shall be vested in an executive committee, to consist of a President, a Vice-President, a Secretary, a Treasurer, and three other members, these officers to be elected by ballot at each annual meeting of the Society to serve one year.

V.

The Annual meeting of the Society shall be held soon after the concluding lecture of the course given during the year, at a time and place to be determined by the Executive Committee. Special meetings may be held at such times and places as the Executive Committee may determine. At all the meetings *ten* members shall constitute a quorum.

VI.

Changes in the Constitution may be made at any meeting of the Society by a majority vote of those present after previous notification of the members in writing.

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THE NEW PHYSIOLOGY *

DR. J. S. HALDANE

University of Oxford

LOOKING back on the history of physiology we can see that there have been various turning-points in general physiological theory, and consequently in the trend of research. Particular discoveries or series of discoveries, often in allied sciences, have led to these turning-points.

The last great turning-point in physiology was about the middle of last century. Up till then it was generally held that in a living organism a specific influence, the so-called "vital force," controls the more intimate and important physiological processes. Inspired by the rapid advances of physics and chemistry, the younger physiologists of that time broke away from vitalism, and maintained that all physiological change is subject to the same physical and chemical laws as in the inorganic world, so that in ultimate analysis biology is only a branch of physics and chemistry.

The subsequent progress of physiology has shown that all, without exception, of the physical and chemical hypotheses then advanced in explanation of intimate physiological processes were far too simple to explain the facts; but the general conclusion that biology is only a special application of ordinary physics and chemistry became firmly established, and is still what may be called the orthodox creed of physiologists. It may be truly said that most physiologists look upon this creed as something which has been established for all time, and that they would be inclined to regard any deviation from it as harmful scientific heresy. Nevertheless I think that we have again reached a turning-point, and that a new physiology is arising in place of the physico-chemical physiology which has held sway for so many years. I propose in this lecture to give some account of how, as it seems to me, this new physiology is shaping itself.

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* Delivered October 14, 1916.

It is natural for us to assume that the aim of all investigations in physiology must be to ascertain the causes of physiological activity. However complex a physiological reaction may be, the conditions which determine it can be investigated experimentally; and from long experience we can be quite certain that such experimental investigation will always lead to some result, however obscure. There is, and can be, no limit to experimental investigation of causes. When, however, we examine the results obtained by experimental physiology there emerges a point in which they differ greatly from the results ordinarily obtained in the investigation of inorganic phenomena: for it is characteristic of physiological reactions that they are dependent to an extreme degree on all sorts of environing conditions. We recognize this when we speak of stimulus and response rather than of cause and effect. When the light from a star is focused on the retina there is a physiological response by night, but none by day. The response evidently depends on the existing state of excitation of the whole retina. It also depends on the normal nutrition of the retina and brain. If the blood is abnormal in composition the ordinary response is interfered with; and we are as yet only at the beginnings of knowledge with regard to the minute changes in blood composition and other conditions of environment which are sufficient to affect the response very materially.

It is the same with every physiological response. The further we investigate the more evident does it become that each physiological response depends on a vast number of conditions in the environment of the responding tissue. On superficial investigation we do not realize this: for we can often get exactly the same response, time after time, with the same stimulus. To the attainment of this result is is only necessary to see that the conditions are "normal." It is only after more thorough investigation that we find that "normal conditions" imply something which is both extremely definite and endlessly complex. We then begin to realize that the maintenance of normal conditions is from the physical and chemical standpoint a phenomenon before which our wonder can never cease.

Physiological investigation of causes seems, thus, to lead us

up to a tangled maze of causal conditions. He who looks for definite "causal chains" in physiological phenomena finds in place of them a network of apparently infinite complexity. The physiologists who led the revolt of last century against vitalism did not see this network. To them it seemed that there were probably simple physical and chemical explanations of the various physical and chemical changes associated with life. The progress of experimental physiology since that time has effectually shown that this was only a dream, and physiologists are now awakening from the dream.

But we are also awakening from another dream. About the middle of last century it seemed as if, in the current conceptions of matter and energy, we had reached finality as regards the inorganic world. The chemical atom, on the one hand, and the energy associated with it, on the other, seemed to represent bed-rock reality—a reality including not merely inorganic, but also organic phenomena. Discoveries connected more particularly with electrical and electro-chemical phenomena, the periodic law, and radio-activity are awakening us from this dream also. The supposed bed-rock reality of a former generation seems to be melting down before our eyes. The solvent has been the study of particular phenomena, such as those of radio-activity. The professional physicists and chemists have hitherto kept away from the serious study of life. For the most part they have regarded life as something apart: or as a complex physical and chemical phenomenon which is not likely to throw any light on the deeper problems of physics and chemistry. In this attitude I think that they have been mistaken; but in any case it is evident that we must guard against the quite unwarranted assumption that the only possibility of advance in physiology is by the direct application to life of the physical and chemical ideas which held unchallenged sway for so many years.

In this reference I should like to reply to some remarks, made partly with reference to my own writings, by my friend Professor Macallum of Toronto, in a very able and interesting presidential address to the American Society of Biological Chemistry two

years ago.[1] After frankly admitting that the apparent difficulties of the mechanistic interpretation of life "put a task upon the human spirit which is apparently not imposed thereon in the theoretic explanation of any other department of science," he proceeds to argue that this is because "our knowledge of the laws that operate in matter is as yet only a very remote approximation to the whole of the lore on this subject that is possibly attainable and that will be ultimately attained." He feels, however, that this defence of the mechanistic theory is somewhat dangerous, and therefore proceeds to point out "that though we know so little of the properties and laws of matter, we know it with a degree of certainty which is not exemplified in the case of any other department of the known or the knowable, and further that the most rational method of interpreting vital phenomena is to explain the unknown in terms of the known, to trace back the causation of the obscure and mysterious to the operations of wholly natural laws and processes."

Now with this latter sentiment I am in entire agreement; but I would point out that Professor Macallum had just invoked not what he considers the known, but, on the contrary, the totally unknown properties of matter, to furnish us with a future physico-chemical explanation of life. I confess that there is in his argument a certain theological smack which strongly appeals to me as a fellow Scotchman. In the domain of "Apologetics" he would, I feel sure, make a great impression. But in the domain of Natural Science we have to examine arguments somewhat closely, and it seems to me that his admissions, which are right and unavoidable, carry him so far that his defence of the mechanistic theory of life is wholly unconvincing. One can not get round the fact that the mechanistic theory has not been a success in the past, and shows no sign of being a success in the future.

When we look broadly at biological phenomena, it is evident that they are distinguished by one universal characteristic. The structure, activity and life history of an organism tend unmistakably to maintain a normal. Accident may destroy an organism, or even a whole species, but within limits of external environ-

[1] *Journal of Biological Chemistry*, XVII., p. VIII., 1914.

ment which are the wider the more highly developed the organism is, the normal life history of each individual is fulfilled.

If, now, we consider the advance of physiological knowledge from the standpoint of the efforts which have been made, not to ascertain the causes of vital activity, but to track out its normal details, the past history of physiology takes on a new aspect. It becomes a record, not of disheartening repulse before a hopeless wire entanglement, but of continuous progress. The new physiology of which I wish to speak to-night is a physiology which deliberately and consciously pursues this line of progress, leaving on one side what one may call the "causal" physiology handed down to us from the last generation. This new physiology is in one sense not new, but very old. It is only new in the sense of consciously pursuing an aim which has nearly always been instinctively pursued by physiologists, and particularly by the great physiologist from whom this society takes its name.

Now I think that many of my hearers will at once say that such a course may be useful up to a certain point, but that it is not true science, and that therefore we can not desert the old attempts. We must, in fact, still continue our frontal attacks on the wire entanglement. To this criticism I shall endeavor to reply later. But meanwhile I should like to explain more clearly, and by means of examples, what the new physiology aims at.

Perhaps I can do this most directly by referring first to the corner of physiology which has largely occupied my own attention—the physiology of breathing.

When we count the breaths, or measure their depth, we find much irregularity, as if there were no very definite or exact regulation of the breathing. Any active occupation, such as speaking or singing, interferes in various ways with the breathing, and the impression at first produced is that the regulation of breathing is very rough. It is also commonly believed that by special training we can increase, or "improve," the ventilation of the lungs. On the other hand it has been well known for long that the breathing is more or less regulated to correspond with the consumption of oxygen and production of carbon dioxide in the body. Thus during heavy muscular exertion greatly increased breathing

accompanies the greatly increased oxidation in the tissues. Another fact, well known to physiologists, is that if the lung ventilation is by artificial or voluntary means greatly increased for a short time, there follows a period of "apnea," during which natural breathing is absent. The exact cause of this apnea was till recently obscure. In 1868 Hering and Breuer showed that the inflation of the lungs in inspiration gives rise to impulses passing up the vagus nerves, and inhibiting further inspiratory impulses from the respiratory center, at the same time starting expiration. Deflation of the lungs in expiration has a converse effect. So long as the vagi are intact they are constantly playing this game of battledore and shuttlecock with the respiratory center, and Hering called this the "self-regulation" (Selbst steuerung) of breathing. The apnea following excessive ventilation of the lungs was interpreted by subsequent physiologists as the summed inhibitory effect of repeated distentions. Fredericq showed, however, that apnea is produced when the respiratory center of one animal is supplied with blood from another animal the lungs of which are excessively ventilated. This, therefore, is a true "chemical" apnea, due to over-aeration of the arterial blood, and was distinguished from "vagus" apnea. Nevertheless the correlation of the various "factors" apparently involved in the regulation of breathing remained extremely obscure.

I observed that when the air breathed is gradually and increasingly vitiated by rebreathing it, or by what is known to miners as "black damp," the breathing is also increased, but not in any simple relation to the extent of the vitiation. With a steady increase in the vitiation the breathing at first increases only a little, but as the vitiation increases further the effect on the breathing is greater and greater. Thus an increase from 4 per cent. to 5 per cent. in the percentage of CO_2 in the inspired air produces about 20 times as great an effect on the breathing as an increase to 1 per cent. from the normal of 0.03 per cent. Observations of this kind suggested that the breathing is so regulated as to maintain a certain normal percentage of carbon dioxide in the air within the lungs, and that as the percentage in the

inspired air rises a greater and greater increase in the breathing is required to maintain this normal. It is, moreover, excess of carbon dioxide that excites the breathing. A corresponding deficiency of oxygen has no such effect.

It was found by Mr. Priestley and myself that a sample of the air in contact with the blood in the lungs could easily be obtained by catching the latter part of the air expired in a deep inspiration. As we expected, the percentage of carbon dioxide in this air turned out to be on an average practically constant for each individual.

If the frequency of breathing is voluntarily varied, even as widely as from three a minute to 60 a minute, the depth adjusts itself so as to keep the average alveolar percentage of carbon dioxide almost absolutely steady; and conversely if the depth is varied. With resistance to breathing there is a similar effect. The effort put into inspiration and expiration is so increased as to overcome the resistance and keep the alveolar carbon dioxide almost steady. If the breathing is temporarily interrupted or abnormally increased, the time is made up afterwards, so that the average alveolar carbon dioxide percentage is practically steady. If, finally, the inspired air is vitiated by carbon dioxide, the breathing is so increased as to keep, if possible, the alveolar percentage approximately steady.

The effects discovered by Hering and Breuer appeared to them to depend simply on the state of mechanical distention of the lungs, and to have no relation to the chemical regulation of breathing. Mr. Mavrogordato and I have quite recently re-investigated these phenomena in man. The results showed that the amounts of inflation or deflation needed to produce the Hering-Breuer effects depend entirely on the chemical stimulus of carbon dioxide. When this stimulus is absent, as in apnea, a very slight inflation or deflation will suffice, so that the breathing is, as it were, jammed during apnea; while if the chemical stimulus is strong it needs a great inflation or deflation to produce the Hering-Breuer effect. The vagi prevent useless prolongation of inspiratory or expiratory effort and consequent waste of time in breathing, or damage to the lung structure.

They also coordinate the discharges of the center with actual inflations or deflations of the lungs. When the vagi are cut the breathing becomes slow, and, as Scott showed, can only imperfectly respond to an increased chemical stimulus, since the frequency can not be increased. The influence of the vagi is entirely in the direction of keeping the alveolar air normal. Perhaps nothing illustrates more clearly the dependence of nervous reactions on more fundamental physiological conditions than the varying response of the respiratory center to the stimulus of inflation or deflation of the lungs.

When excessive ventilation of the lungs is so arranged that there is no fall in the alveolar percentage of carbon dioxide, no apnea follows. There is thus no such thing as the so-called vagus apnea. Apnea is simply due to excessive removal of carbon dioxide from the alveolar air.

When the barometric pressure is varied it becomes evident that the normal which dominates the regulation of breathing is not the percentage of carbon dioxide in the alveolar air, but the partial pressure or molecular concentration. At the normal atmospheric pressure of 30 inches there is about 5.6 per cent. of carbon dioxide in the alveolar air, but only 2.8 at 60 inches barometric pressure, and 1.4 at 120 inches. In these three cases the percentage of CO_2 varies widely, but the partial pressure is the same. It is only with constant barometric pressure that the normal percentage is steady.

When the breathing is increased by excess of CO_2 in the inspired air, or increased production of CO_2 in the body, there is, as might be expected, a slight rise in the alveolar CO_2 percentage. It is this slight rise that is the stimulus to increased breathing. Roughly speaking, a rise of 0.2 per cent. increases the resting breathing by 100 per cent., while a fall of 0.2 per cent. produces apnea. The stimulus of the increased CO_2 percentage is conveyed to the respiratory center by the blood. Under ordinary average conditions the center responds with normal breathing when the blood leaving the lungs is saturated with air containing 5.6 per cent. of CO_2, but does not respond at all when the blood is saturated with 5.4 per cent. of CO_2 or less.

The threshold value of CO_2 is, however, greatly lowered by excessive administration of acids or in any condition of so-called acidosis, and is raised by alkalies or an alkaline diet. This and other evidence points to the fact that CO_2 acts on the respiratory center in virtue of its acid properties when in solution.

According to modern ideas the acidity or alkalinity of a liquid depends on its hydrogen ion concentration. The accurate measurement of the hydrogen ion concentration of blood by the electrometric method is attended with great difficulties; but these have been to a large extent overcome by Hasselbalch, of Copenhagen, who has obtained measurements of the effects of saturation with different partial pressures of CO_2 on the hydrogen ion concentration of blood. He has also shown experimentally that when the alveolar CO_2 threshold is lowered or raised by an acid or alkaline diet this raising or lowering is just sufficient to keep the hydrogen ion concentration of the arterial blood sensibly steady. It is now certain, therefore, that what the respiratory center is reacting to when it reacts to a slight increase in the alveolar CO_2 percentage is the consequent slight increase in the hydrogen ion concentration of the blood.

The latter increase is so minute that it can only be detected electrometrically when it is of sufficient extent to produce very gross changes in the breathing. The respiratory center is enormously more delicate as an index of change in hydrogen ion concentration of the blood than any existing physical or chemical method.

As already remarked, the alveolar CO_2 percentage is extremely steady under ordinary resting conditions. This implies that the hydrogen ion concentration of the blood is regulated with almost incredible delicacy, and must be so regulated apart altogether from the breathing. The breathing simply regulates the rapid disturbances in hydrogen ion concentration caused by variations in the production of CO_2: other disturbances are regulated otherwise than by the breathing. There is clear evidence that both the kidneys and the liver play a part in this regulation; but of the marvellous accuracy of the regulation physiologists

had, till the recent work on the physiology of breathing, no clear conception.

It is not merely the hydrogen ion concentration of the blood that is accurately regulated, but also its capacity for taking up a constant amount of CO_2 in presence of a constant partial pressure of this gas. This capacity depends on the concentration of and balance between alkaline salts and albuminous substances in the blood. Recent investigations by Christiansen, Douglas and myself have shown that this concentration and balance are so accurately maintained day by day, and month by month that under normal conditions no deviations can be detected by the most delicate existing method of blood gas analysis. The balance can be temporarily upset by what may be called violent means; but within an hour it is back again to normal. It is, of course, evident that if the carrying-power of blood for CO_2 did not remain normal the breathing and circulation would not, without special adjustment, remain normal.

Now let us look back for a moment, and see where we now stand. The experimental study of the physiology of breathing has led us to the discovery of four normals, the maintenance of which furnishes the interpretation of a mass of what would otherwise be isolated and unintelligible observations. We have first of all the normal alveolar CO_2 pressure. This turns out to be directly subordinate to the normal regulation of the hydrogen ion concentration of the blood, the normal reaction of the respiratory center to hydrogen ion concentration, and the normal regulation of the capacity of the blood for carrying CO_2. With the discovery of each of these normals we have obtained deeper and deeper insight into the physiology of breathing. We have done this, not by merely seeking for causes in the physical sense, but by seeking for interconnected normals and their organization with reference to one another and to other organic normals. These normals represent, not structure in the ordinary physical sense, but the active maintenance of composition. We may fitly call this living structure, since so far as we know all living structure is actively maintained composition, the atoms and molecules entering into which are never the same from moment to

moment according to the ordinary physical and chemical interpretation. Our method has thus been essentially the same as that of the anatomist who seeks for the normal—the type—which runs through and dominates the variety of detail which he meets with, and who reaches more and more fundamental types.

I wish, now, to point out that the same method has been applied, and is being applied, to other departments of physiology, even though the physiologists applying it may have failed to realize the far-reaching significance of their results.

I will refer first to the general physiology of the blood. The facts that the hydrogen ion concentration and capacity for carrying CO_2 are very accurately regulated in the blood are no isolated facts in physiology, although the accuracy of our physiological means of measurement renders them peculiarly striking. Claude Bernard, in his *Leçons sur les phénomènes de la vie,* was, I think, the first to point out clearly that the composition of the blood, as well as its temperature, is physiologically regulated. He was led to this conclusion more particularly by his observations that in prolonged starvation there is still sugar in the blood, and that even when great excess of sugar is introduced into the body the percentage in the blood remains very steady, as excess is taken up by the liver and other organs, or excreted by the kidneys. Voit's observations on the relative constancy of the sodium chloride in the blood, and the manner in which the kidneys regulate this percentage are of a similar character. If food freed from chloride is administered the elimination of chloride by the urine diminishes to almost nothing, though the high percentage of chloride in the blood-plasma remains about the same. As Voit also showed, the blood during prolonged starvation retains its normal composition, and its volume remains proportional to body weight, while other tissues (*e. g.,* muscle) are reduced.

Dr. Priestley and I have recently investigated the excretion of water by the kidneys. By simply drinking large quantities of water one can produce an enormous increase in the secretion of urine, and this urine is almost pure water. What we wished to observe was the degree of watering down of the blood which

was necessary to produce the huge increase in excretion of water. We did not doubt that the watering down would be very small, but when we attempted to measure the dilution by determining the percentage of hemoglobin we found that there was no dilution at all, though the method used was one of extreme accuracy. When, however, the plan of measuring the electrical conductivity of the serum was adopted, a slight, but quite distinct, diminution in the conductivity could be detected during, and ending with, the diuresis. This showed that there was a slight diminution in the salt-concentration, and to this diminution the secreting cells were reacting. Here, then, we are in presence of another exactly but elastically regulated normal, the slightest deviation from which produces, in the kidneys, a reaction comparable in its exquisite delicacy with the reaction of the respiratory center or liver or kidneys to a change in hydrogen ion concentration.

The physiology of the kidneys has, in accordance with prevalent physiological conceptions, been attacked from the side of "causal" explanation. I know nothing more hopeless than the attempts to explain the outstanding features of secretion of urine on the lines of ordinary physics and chemistry. So far as the facts are yet known we can, however, get a practical grasp of the kidney activities if we attack the subject from the standpoint of the active maintenance of the normal blood composition.

Let me turn now to the general physiology of nutrition. In the preliminary stages of investigation of this subject physiology has owed much to the pure chemists, and this debt is constantly increasing. We have only to think of the work of such men as Black, Priestley, Lavoisier and Liebig. Like Wöhler, who synthesized urea, Liebig was a convinced vitalist. For him there was a central kernel of vital activity under the control of the "vital force"; but outside this central kernel he interpreted the phenomena of nutrition on purely chemical lines. He thought of oxygen as something free to oxidize anything oxidizable within the body, except what is protected by the vital force; and he assumed that the greater the concentration of oxygen in the lungs, and the greater the supply of food-material to the body,

the greater will be the amount of oxidation, since only a limited amount of oxidation is under the direct control of the vital force. He gave special attention to the elimination of urea and other products of nitrogenous oxidation, and introduced methods of measuring the nitrogenous waste. It was found, apparently in direct confirmation of his general ideas, that the amount of urea excreted rises and falls, except for a certain starvation minimum, in direct proportion to the amount of albuminous food eaten. The excess over the starvation minimum was looked upon as "luxus consumption"—an ungoverned oxidation, due to simple chemical factors.

But the matter was soon carried further by the physiologists—particularly by Pflüger, and by Voit and his pupil Rubner. It was found that, other conditions being equal, the consumption of oxygen is within wide limits independent of the abundance of its supply, and that the actual consumption of oxygen per unit of body weight is very little different during starvation from what it is when abundant food is supplied. In starvation more fat is being oxidized to compensate for the deficiency in albuminous oxidation. Finally, the brilliant work of Rubner established the fundamental fact that within very wide limits different food substances are simply substituted for one another within the organism in direct and exact proportion to the energy which they furnish when broken down. The energy liberation per unit body weight is practically constant, but if excess of food is taken the excess of potential energy is stored up as fat and glycogen, while if food is withheld the stored excess is used up. Even when all the stored fat and glycogen is used up, the organism finally flings its own living structural substance into the balance, and in this last desperate effort to maintain the normal metabolism the nitrogenous oxidation again rises to an amount which for a short time compensates for the energy previously yielded by fat. When death from starvation at length comes the old flag—the flag of life—is still flying.

The massive work of Atwater and his pupils on human nutrition, in which it was shown that the normal daily food requirement of a man is about 3,500 calories in energy-value, was of

course a direct extension of the idea of normal nutrition. We maintain an energy consumption of about 3,500 calories, just as we maintain about 5.6 per cent. of CO_2 in our alveolar air, or hemoglobin of 18.5 per cent. oxygen capacity in our blood, or legs of a certain length and anatomical structure. By a strange confusion of ideas the idea is abroad that nutrition is a matter of simple chemistry and physics, and that when we estimate food values in calories, we are exemplifying this fact. This is enough to make a staunch old vitalist like Harvey or Johannes Müller turn round in his grave and laugh. What is it in the body that measures out or withdraws protein, carbohydrate and fat with meticulous accuracy in terms of their energy value, in such amount as to maintain the normal energy metabolism? Is it not the vital spirit or vital force? the old physiologists would ask. Is not this phenomena of a piece with all the other distinctive phenomena of life, and ought not physiology to face these phenomena fairly and squarely and generalize from them, not run away from them? This is the question I am trying to put to you now.

Now I wish to make it clear that it is not vitalism, but simply biology, that I am preaching. Vitalism is a very roundabout and imperfect attempt to represent the facts. Physiological study, and biological study generally, seems to me to make it clear that throughout all the detail of physiological "reaction" and anatomical "structure" we can discern the maintenance of an articulated or organized normal. This idea brings unity and light into every corner of physiology. In other words, it helps us within limits to predict, just as the ideas of unalterable mass and energy help us within limits to predict, or the ideas of time and space help us within limits to predict. I claim nothing more for it, but also nothing less. The idea of life is just the idea of life. One can not define it in terms of anything simpler, just as one can not define mass or energy in terms of anything simpler. But this one can say—that each phenomenon of life, whether manifested in "structure" or in "environment," or in "activity," is a function of its relation to all the other phenomena, the relation being more immediate to some, and less so to others.

Life is a whole which determines its parts. They exist only as parts of the whole.

At first sight it might seem as if it must be very difficult to make use of this conception as an instrument of research: for evidently we can not investigate the parts without investigating the whole. The difficulty is only apparent. The whole is there, however little we as yet comprehend it. We can safely assume its presence and proceed to discover its living details piece by piece, in so doing adding to our knowledge of the whole. If, on the other hand, we attempt to take the organism to pieces, or separate it from its environment, either in thought or in deed, it simply disappears from our mental vision. A living organism made up of matter and energy is like matter and energy made up of pure time and space: it conveys to us no meaning which we can make use of in interpreting the facts.

But is there not matter and energy in a living organism? Do we not assume this at every step in physiology? We make use of the ideas of matter and energy in biology, just as the physicist makes use of the idea of extension in the investigation of matter. To the biologist, however, the structure and activity of an organism are no mere physical structure and activity, but manifestations of life, just as to the physicist the extension of matter is no mere mathematical extension, but a manifestation of the properties of matter, with a physical and not a mere mathematical meaning. This is the answer to those who point to the dependence of physiology on physics and chemistry, and conclude from this that physiology can not be anything but a department of physics and chemistry. By a similar chain of reasoning physics would be nothing but a branch of mathematics, and mathematics itself would melt away into that universe of unconnected "impressions" which David Hume imagined, but Immanuel Kant showed to be non-existent.

The limits of time prevent my giving further examples of the light which the conception of the normal throws on the details of every part of physiology, and I must now try to probe more deeply. It may be pointed out that although it is useful in matters of detail to bear in mind that a living organism tends

to maintain a normal of both structure and activity, and to pass through a normal life history, yet in ultimate analysis all this *must* be due simply to the reactions between its structure and physical and chemical environment. I will not at this point quarrel on general grounds with the "must," but simply endeavor to test it by the facts of physiology.

We can distinguish in a living organism what seems a more or less definite structure of bony matter and connective tissue. Yet we know that all this is built up, and in adult life is constantly being pulled down, rebuilt and repaired, through the activities of living cells. It is thus within the living cells that we must look for the structure which is supposed to react so as to maintain the normal. These cells are made up of what has been called "protoplasm." Now the more we study protoplasm the more evident does it become that this "substance" is extraordinarily sensitive to the minutest changes in environment. Take away or diminish or increase the minute traces of calcium or potassium salts in the blood-plasma, or the traces of various substances supplied to the blood by other organs; or add traces of certain other substances: the reactions of the protoplasm are quickly altered, and its structure may be destroyed. It is evidently in active relation with its environment at every point, and one can not suspend this activity without altering it. Even deprivation of oxygen for, perhaps, a minute may kill a nerve-cell. There is no permanent physical structure in the cell: the apparent structure is nothing but a molecular flux, dependent from moment to moment on the environment.

Now when we look at the blood, the internal or immediate environment on one side of the cells in the body, we find, as already shown, that this is almost incredibly constant in composition. Were it not so the reactions of the cells would become chaotic, and their structure would be completely altered if not destroyed. But the constancy of the blood is maintained by the combined reactions of the organs and tissues themselves. The intimate structure of the living cells depends on the constancy of the blood, and the constancy of the blood depends on the intimate structure of the tissues. If we regard this condition as

simply a physical and chemical state of dynamic balance, it is evident that the balance must be inconceivably complicated and at the same time totally unstable. If at any one point in the system the balance is disturbed it will break down, and everything will go from bad to worse.

A living organism does not behave in this way: for its balance is active, elastic, and therefore very stable. When a disturbance affects its structure or internal environment it tends to "adapt" itself to the disturbance. That is to say its reactions become modified in such a manner that the normal is in essential points maintained. An injury heals up: destroyed tissue is reproduced, or other parts take on its function: the attacks of microorganisms are not only repelled, but immunity to future attacks is produced. In reproduction the body periodically proceeds to renew almost the whole of its structure. Death may be regarded as a periodical scrapping of structural machinery, and reproduction as its complete renewal.

The Anglo-American expedition of which I was a member studied, on the summit of Pike's Peak, Colorado, adaptation to the want of oxygen which causes, in unadapted persons, all the formidable symptoms known as "mountain sickness." As adaptation proceeded the blueness of the lips, nausea, and headache completely disappeared, and it was then found that even during the rest the lung epithelium had begun to secrete oxygen actively inwards. The kidneys and liver were now also regulating to a lower degree of alkalinity in the blood, so that the alveolar CO_2 pressure was diminished, and the breathing consequently increased, thus raising the oxygen-supply to the lungs. There was also a marked increase in the hemoglobin percentage and in the blood volume. The organism had so adapted itself as nearly to compensate for the deficiency in oxygen supply, just as a heart gradually compensates for a permanent valvular defect.

The normals of a living organism are no mere accidents of physical structure. They persist and endure, and they are just the expression of what the organism is. By investigation we find out what they are, and how they are related to one another; and the ground axiom of biology is that they hang together and

actively persist as a whole, whether they are normals of structure, activity, environment or life history. In other words organisms are just organisms and life is just life, as it has always seemed to the ordinary man to be. Life as such is a reality. Physiology is therefore a biological science, and the only possible physiology is biological physiology.[2] The new physiology is biological physiology—not bio-physics or bio-chemistry. The attempt to analyze living organisms into physical and chemical mechanism is probably the most colossal failure in the whole history of modern science. It is a failure, not, as its present defenders suggest, because the facts we know are so few, but because the facts we already know are inconsistent with the mechanistic theory. If it is defended it can only be on the metaphysical ground that in our present interpretation of the inorganic world we have reached finality and certainty, and that we are therefore bound to go on endeavoring to interpret biological phenomena in the light of this final certainty. This is thoroughly bad metaphysics and equally bad science. It is the idea of causation itself that has failed, and failed because it does not take us far enough. We have not at present the data required in order to connect physical and chemical with biological interpretations of our observations; but perhaps the time is not far off when biological interpretations will be extended into what we at present look upon as the inorganic world. Progress seems possible in this direction, but not in the direction of extending to life our present every-day causal conceptions of the inorganic world.

I now wish to add a few words as to the relation of physiology to medicine; for I am one of those with an intense belief in the intimate connection between the two sciences, and it seems to me that the mechanistic physiology of the nineteenth century has failed to take the rightful position of physiology in relation to medicine. What is the practical object of medicine? It is to

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[2] It has been suggested to me that if a convenient label is needed for the teaching upheld in this letter the word "organicism" might be employed. This word was formerly used in connection with the somewhat similar teaching of such men as Bichat, von Baer and Claude Bernard. *Cf.* Delage, L'Hérédité, Paris, 1903, p. 436.

promote the maintenance and assist in the reestablishment of health. But what is health? Surely it is what is normal for an organism. By "normal" is meant, not what is the average, but what is normal in the biological sense—the condition in which the organism is maintaining in integrity all the interconnected normals which, as I have already tried to indicate, manifest themselves in both bodily structure and bodily activity.

Now for the mechanistic physiology there are no interconnected normals, just as in the inorganic world as at present interpreted there are also no interconnected normals. If we look through an average existing text-book of physiology we find a great deal about the effects of this or that stimulus, a great deal also about the external mechanism and chemistry of bodily activity—a great deal, in other words, about what lies on the surface but never takes us further. Along with this there are perhaps also some inconclusive discussions of the possible mechanism of such processes as physiological oxidation, secretion, growth, muscular contraction, or nervous activity. Very little will, however, be found about what in reality lies still more on the surface, but also penetrates deep down—the maintenance within and around the body of normal organized structure and activity. The maintenance of the normal is something for which there is no place in the mechanistic physiology, since acording to this physiology maintenance must be in ultimate analysis only an accident of structure and environment—a fitful will o' the wisp which does not concern true science.

But medicine, as we have seen, is supremely interested in the physiological normal. What a man sees at the bedside is a perversion of the normal, and nature's attempts to restore it, with what assistance medicine can give. For medicine it is necessary to know the normal in its elastic and active organization. He who knows how the body regulates its normal temperature will not confuse heat-stroke with fever, or make the mistake of attributing fever to mere increased heat-production in the body. He who knows how the breathing is normally regulated will be in a position to distinguish at once between various causes of abnormal breathing; and similarly for every abnormal symptom

met with in disease. But the mechanistic physiology gives a minimum of information about the regulation of the normal. One looks in vain in physiological text-books for connected accounts of the regulation of breathing, circulation, kidney activity, general metabolism, nervous activity. The main facts of physiology are partly ignored, and partly strewn about in hopeless disconnection and confusion. A student of medicine may learn some true physiology at the bedside, or he may never learn it at all, and become either a hopeless empiric or what I do not hesitate to call a mechanistic pedant.

Medicine needs a new physiology which will teach what health really means, and how it is maintained under the ordinarily varying conditions of environment. We also need a pathology which will teach how health tends to reassert itself under totally abnormal conditions, and a pharmacology which will teach us, not merely the "actions" of drugs, but how drugs can be used rationally to aid the body in the maintenance and reestablishment of health. The new physiology, new pathology, and new pharmacology are growing up around us just now. I can see them more particularly in the splendid advances which the medical and other biological sciences are making in America. You have the advantage of having less of old intellectul machinery to scrap than we have in the old countries; but perhaps we shall not be much behindhand.

If we look on pathology as simply the description of damage to bodily structure, and the analysis of the causes of this damage, then pathology may be very helpful in preventive medicine, but does not help much in therapeutics. When, however, pathology studies the processes of adaptation to the unusual, defence of the organism against the unusual, and reproduction of the normal, just as the new physiology studies the maintenance of the normal under ordinary conditions, then therapeutics and surgery will be aided at every step by pathology, and a rational biological pharmacology will have its chance.

Sometimes one hears the complaint that the world has grown old: that the great discoveries have all been made; and that nothing is left to us now but to work out matters of sheer detail.

Perhaps the great and constantly growing mass of rather uninteresting, but otherwise apparently meritorious scientific literature, increases this impression. At certain moments one may long for the past centuries when there was much less to read, and people seemed to have plenty of time to think, and to have endless material for new discoveries and projects. But in reality I do not think that there was ever more scope for new ideas and discoveries than there is at present. Among the new ideas are those of the new physiology, the outlines of which I have tried to trace in this lecture. Those who do not feel inclined to accept this new physiology, or who are still sceptical as to its theoretical basis, will, I hope, at least make allowance for any personal failure on my part to present it to them in a more convincing form.

THE RÔLE OF FAT IN DIABETES*

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THE work with diabetes at the Hospital of the Rockefeller Institute began with feeding experiments on partially depancreatized dogs, and since then has grown in various directions. The results have been applied in the treatment of human cases, and this side of the investigation has been taken up by Drs. Stillman and Fitz, Dr. Stillman having studied especially the carbon dioxide changes in alveolar air and blood and Dr. Fitz the acetone bodies in blood and urine. It was a great good fortune when Dr. DuBois consented to determine the respiratory metabolism of certain patients at the Russell Sage Institute, and thus (in connection with the similar findings of Benedict and Joslin) some facts were established which were important for the intelligent application of the clinical treatment, and some theoretical questions decided and some others opened up. On the side of the animal experiments, Dr. Palmer has carried out a research in the practically unknown field of the sugar-content of the tissues under normal and various pathological conditions. Dr. Perlzweig and Miss Wishart are assisting in several problems, comprised chiefly under the topic to be discussed. The combination of animal and clinical work is very advantageous, each throwing light on the other. Also, the animal experiments are different from the customary, in that they do not consist in brief observations limited to a single point, but, on the contrary, animals are brought into the desired diabetic or other condition, and then are studied like human patients, through months and years if necessary. This plan has always appeared to me as indispensable for real progress in certain aspects of this problem. Acute experiments cannot give the best picture of chronic disease. Chronic

* Delivered November 4, 1916.

conditions in animals need to be studied by the same combination of clinical, chemical, and microscopic methods as used for human patients, and for the same reason, namely, that one part of the picture can be understood only in relation with the other parts. On this plan, information is gained by determining the means to produce in animals the conditions occurring spontaneously in patients, and then by studying these conditions with the freedom and accuracy which are possible in animal experiments.

The problems of diabetic and normal metabolism are being opened up with surprising rapidity as methods become available. The monograph concerning my research at Harvard was written in 1912, and at that time comparative analyses at frequent intervals as shown in these charts were impossible, because the methods then existing required too much time or material. The present work was done entirely with methods published by American chemists within these few years. The numerous blood-sugar analyses were made possible by the method of Lewis and Benedict, which was used unmodified as originally described. The analyses for acetone bodies were carried out first with a modification of the methods of Shaffer, Marriott, and Folin and Denis, and later by the recent Van Slyke method. The alkaline reserve of the plasma was estimated by Van Slyke's simple and accurate device of the carbon dioxide combining power, which has proved its usefulness both experimentally and clinically. The blood-fat was determined by Bloor's method in the modification employed by Murlin and Riche. The methods introduced by Sellards and by Levy, Rowntree and Marriott deserve mention in connection with the study of acidosis, but had to be omitted in this research. It is evident that various blood and urine examinations, calorimetric studies, tissue analyses, and histologic and other investigations are most instructive when performed not upon different animals but upon the very animals for which these other data exist.

It has been decided to discuss the rôle of fat in diabetes because of its theoretical and practical importance, and because it has constituted one of the most confused and perplexed phases of the subject, where any light from any source may be deemed

desirable. The feeding experiments mentioned at the outset have included the feeding of fat, and this has involved the longest and most difficult experiments of the series. Therefore, this opportunity is taken to present the results of some of the experiments in this direction, and this paper will consist largely of observations not heretofore published.

It was formerly impossible to make a satisfactory study of this question with animal experiments because only two types of diabetic animals were known, namely, the Minkowski type with total extirpation of the pancreas, and the Sandmeyer type with removal of most of the pancreas and isolation of the remainder from its duct communications, so that the blocking of secretion brought on sclerosis and atrophy. Neither of these types of animals is capable of digesting and absorbing enough fat for the purpose, or of affording in other respects a sufficiently close reproduction of human diabetes, where there is ordinarily no deficiency of pancreatic digestion. I have previously described a method which gives a good approximation of the clinical condition. Those familiar with the publication will recall that this consists in removing most of the pancreas, leaving a remnant ordinarily of one-eighth to one-twelfth always communicating with a duct. With the smaller remnants the diabetes is severe; with the larger remnants it is mild; but by feeding the animals beyond their tolerance, so as to maintain a prolonged glycosuria, there is progress downward, as in human cases, and the mild diabetes becomes as severe as that which follows the more extensive removal of pancreatic tissue. For our present purpose and for most purposes the best results are obtained by having the pancreas remnants as large as possible, for two reasons. One is the avoidance of cachexia. The totally depancreatized dog dies within a relatively brief period, while an equal loss of sugar and nitrogen caused by phloridzin is far better borne. Some dogs with very small pancreas remnants do fairly well, but a large proportion of them fail to thrive, gradually emaciate, and die; whereas dogs with larger remnants but equally severe diabetes thrive much better. Nothing is known concerning the nature of this peculiar pancreatic cachexia; presumably it repre-

sents metabolic failure. The second reason referred to consists in the power of digestion. The digestion of the partially depancreatized dogs is never quite equal to the normal. Some of the diets used tax the digestion of normal dogs. In particular, the larger the pancreas remnant the better do the dogs dispose of a high fat diet. Occasional dogs become diabetic with exceptionally large pancreas remnants, and such animals are valuable for this use. In general, the program is to choose young dogs as strong and as voracious as possible. The pancreatic tissue removed is the minimum required to produce mild diabetes. The tolerance is then broken down by overfeeding, the diet sometimes including several hundred grams of glucose daily until the desired degree of diabetes results. The animals are then often kept free from glycosuria for several weeks or months, and any tendency to recovery of too high a tolerance is checked by a period of overfeeding. In the best cases there is thus a decided hypertrophy of the acinar tissue, so that the remnant may come to equal as much as one-fourth of the original weight of the pancreas, and yet the diabetic condition is maintained. Such dogs have highly satisfactory vitality and digestive power, and are very well suited for the fat feeding and other experiments. Though the internal secretory function has thus been injured largely by functional means, it remains fairly constant at its low level, and any recovery of tolerance is exceedingly slow. In this respect the animals resemble human patients. There is a difference in that the functional overstrain in dogs results in actual anatomical destruction of cells in the islands of Langerhans, while such an anatomical effect in human patients is still doubtful.

The rôle of fat in diabetes will be discussed in its relation to three subjects.

I. LIPEMIA.

The first of these is lipemia. Here we deal with the disposal of fat from its absorption by the bowel to its taking up by the cells of the body. A few words may be devoted to the normal process, which is still obscure in essential points. An early dispute concerning digestion has been settled, since it is established

that fat is not absorbed in emulsion as such but only as the split products. In the intestinal epithelium the glycerin and fatty acids are recombined into neutral fat. Some earlier researches, especially those of Rosenfeld and others in the dispute over fatty degeneration and infiltration, led to the view that this recombined fat is identical with the food-fat; that is, that only the fat synthesized from carbohydrate or other foods can be peculiar to the species, while otherwise the fat of the body takes its character from the fat of the food. A series of authors, Bloor being the latest, have modified this extreme view, and have shown that the epithelium changes and rearranges the constituents to considerable extent, so that the recombined fat differs from the food-fat in being more nearly like the natural fat of the animal. The procedure of splitting and recombination therefore apparently serves for the absorption of useful fats, the exclusion of non-saponifiable substances such as mineral oils, and the partial modification of the absorbed fat to resemble the specific body-fat. Some of this recombined fat is perhaps taken up in the blood capillaries and carried in the portal circulation to the liver. But at least 60 per cent. of it is known to enter the lacteals and pass in fine emulsion through the thoracic duct into the systemic circulation. Obviously, it canot linger long in the blood, which would be hopelessly overloaded by the fat of a single meal. The cells remove it rapidly, so that, nothwithstanding the heaviest intake, the blood-fat like the blood-sugar varies only within narrow limits. In the phraseology of Magnus-Levy, the level of the blood-fat must represent the balance between inflow and outflow at any given time. This brings us to the consideration of the fat content of normal blood.

There have been described three physical forms in which fat may exist in the blood. First may be mentioned the occult form, which ordinarily predominates. The fine emulsion of the chyle is changed as it enters the blood stream. The droplets apparently dissolve, so that the clear blood plasma contains fat which cannot be colored by osmic acid or any fat stains or extracted by ether or other solvents, and can only be demonstrated by digesting the proteins with enzymes, acid, or alkali, or precipitating them with

reagents such as alcohol. This fat is probably non-dialyzable and seems to exist in some colloid combination. The second form consists of surplus or less soluble fat, in microscopic droplets, the blood-dust or hemoconia. These can be stained with fat stains and extracted with fat solvents, and as they increase they make the plasma first turbid and then milky, as in digestion lipemia. The third possible form of fat is perhaps never normal. It is the form described by several authors when the plasma is cloudy or even opaque, yet cannot be cleared by the centrifuge; tiny droplets may or may not be visible under the microscope, but the substance is not colored by fat stains or dissolved in ether or chloroform. Boggs and Morris reported over 4 per cent. of this form of fat in the blood in anemic lipemia of rabbits. Bloor found that this form arises *in vitro* when certain abnormal (viz., diabetic or anemic) bloods are allowed to stand, the previously clear plasma becoming turbid. The three physical forms are presumably due to varying proportions and combinations of fats and lipoids among themselves, and possibly with proteins, salts, or other substances. Normal fasting plasma is clear and, according to Bloor, contains approximately 0.29 to 0.42 per cent. fat in human beings, and somewhat more, viz., 0.51 to 0.66 per cent. in dogs. Turbidity may appear within about an hour after a fat meal; it reaches its maximum in about six hours, and after twelve hours the plasma is again clear. The susceptibility to alimentary lipemia, in other words the balance between the digestive function and the assimilative function, differs in different species. Nothing is known concerning differences in the rate at which their tissues can take up fat. Rabbits are resistant to alimentary lipemia, and increasing the dose of fat merely causes diarrhea (Neisser and Bräuning). Sakai proved that rabbit blood has no unusual capacity for carrying concealed fat. Therefore it is possible to accept the explanation of Kreidl and Neumann, based on dark-field examinations of hemoconia, that intestinal absorption of fat is much slower in herbivora than in carnivora; but the question has not been investigated chemically, and offers a promising opportunity for the use of the new micro-methods. The goose has such a digestive power that it can be enormously fattened by

forcible feeding; and in the serum of such stuffed geese Bleibtreu, also Hervson and Sedel, found some 6 per cent. fat, though the rye used in Bleibtreu's feeding contained only 1.5 to 2.5 per cent. fat. Stuffing geese with fat-free food did not cause lipemia. Man and the dog are intermediate between these extremes. Alimentary lipemia between 1 and 2 per cent. is probably the highest that ever occurs in normal human subjects. Munk and Friedenthal found as high as 3 per cent. alimentary lipemia in dogs. It is impossible to produce in dogs a lipemia equal to that of the stuffed goose by any quantity or duration of fat feeding, for the digestion breaks down before any such plethora is produced in the metabolism.

Thus far the word fat has been used in the old-fashioned sense, indicating the whole of the ether-soluble constituents. But aside from the neutral fat, which preponderates, and the soaps and free fatty acids possibly occurring in small quantities, there are constantly present the substances called lipoids, grouped under the titles lecithin and cholesterol. Such phosphatids and sterols exist in every living cell; they are evidently of the most indispensable importance somehow, but their function is practically unknown. Even the gross metabolism of lecithin and cholesterol is mostly unknown. They can be absorbed as such from the bowel and also synthesized by the body. Cholesterol especially is excreted through the bile and feces. The nervous system is rich in these substances, and the liver, the red corpuscles, and the adrenal cortex have all been claimed to play a part in their metabolism. Bloor finds that the lecithin and cholesterol are each about a third of the total ether-soluble extract of normal blood. Their ratio is fairly constant and remains so in most pathological conditions, suggesting an important relationship. The quantity of glycerides in the fasting plasma might possibly be zero, which would mean that the entire transport of fat would be in the form of lecithin and cholesterol esters; but a small quantity of simple fat is probably present. Digestion of fat brings an increase in the glycerides.

Both lecithin and cholesterol rise in most forms of lipemia. Lecithin is markedly increased in alimentary lipemia even though

the ingested fat be practically free from lecithin. Terroine, Hagenau and others observed a similar parallelism between fat and cholesterol even when the food fat was extremely poor in cholesterol; and Sakai laid down the rule that there is no lipemia without cholesteremia, supposedly because of the solubility of cholesterol in fat; and Gardner and Lander have pointed to an absorption of cholesterol from the bile; but Bloor has found no change in the cholesterol during alimentary lipemia. The occurrence of the lipoids free or in esters or other compounds may have special significance, but this is unknown at present.

The relations in plasma and corpuscles also deserve notice. Normally, according to Bloor, lecithin and cholesterol are nearly equal in the plasma; the lecithin content of the corpuscles is approximately double that of the plasma, and also double the cholesterol of the corpuscles. Bloor assumes that two-thirds of the plasma cholesterol is combined in esters. It is believed that all the cholesterol of the corpuscles is free. The corpuscles contain no true fat or only indeterminable traces.

Connstein claimed that the presence of red corpuscles is necessary in order for the fat to be changed from the emulsified to the soluble form. Munk and Friedenthal asserted that the corpuscles actively take up fat, so that in alimentary lipemia they may contain a higher percentage than the plasma. Bloor has confirmed this statement, and furthermore has found that lecithin increases chiefly in the corpuscles and only slightly in the plasma. Following up the idea of Loew and of Leathes that all fat is utilized *via* lecithin, Bloor has set up the hypothesis that all or most of the absorbed fat must be taken into the corpuscles and converted into lecithin before it can be assimilated. He finds here an explanation of the arrangement whereby fat is led into the general venous circulation to be thoroughly mixed with the blood before being carried to the liver or any other capillary domain for assimilation. Since lecithin is increased in a brief lipemia, as during digestion, he assigns special importance to it in the early stages of assimilation; and as cholesterol is increased in lipemias of long standing, he ascribes importance to it in the later stages of assimilation of fat.

The actual mechanism by which cells take up fat is unknown. Authors from Hanriot to Rona and Michaelis, Caro and Sakai have undertaken to demonstrate a lipase function. The process in assimilation would thus correspond to that in digestion. But the alleged lipase is so feeble that its presence or activity is hard to determine accurately by titration, and recourse is had to delicate physicochemical tests with the stalagmometer. Also, it seems truly characteristic of the subject of diabetes that the attempt should be made to explain lipemia by diminution of lipase, disregarding the extreme diminution of lipase in diseases without lipemia, as reported, for example, by Bauer. The lipolytic enzyme of blood seems classifiable with the glycolytic enzyme as accidental and unimportant, and the notion of enzymatic digestion of fat in the blood plasma or at the cell boundary does not merit serious consideration. On the other hand the conversion of fat into the plasma-soluble form, through the process supposed by Bloor or any other process, appears as a significant phenomenon. All living cells contain a similar "masked" or combined fat, and the most plausible view is that fat passes through the cell boundaries in this colloid or soluble form. Taylor refers to this as the "metabolic" form of fat. Nevertheless, it must be borne in mind that we are dealing with hypotheses throughout, and that nothing is known positively concerning the means by which the cells take up fat from the blood.

Except after meals it seems probable that hyperlipemia is abnormal like hyperglycemia. A slight lipemia accompanying exercise was observed by Murlin and Riche. Bloor suspects that excitement may increase blood-fat as it does blood-sugar. Animals possessing stores of fat show a slight lipemia during fasting, supposedly because of increased transport of fat. Bloor's highest figure was about 0.9 per cent. Slight lipemia may occur in pregnancy, but according to studies by Klinkert and others, cholesteremia is the most prominent feature, and is responsible for the occasional xanthelasma and possibly related to the accompanying hypertrophy of the adrenal cortex. Just as hyperglycemia, so also hyperlipemia may occur in various metabolic disorders. It may be found in obesity, alcoholism, and nephritis. Chauffard

and Grigaut described hypercholesteremia in nephritis. According to Lauber and Adamuck, Zinsberg and Chauffard the white flecks in the retina in nephritis are largely accumulations of cholesterol esters, and (Borberg, Landau) cholesterol is increased in the adrenal cortex. The familiar accompaniments of nephritis, namely, atheroma of blood-vessels and the arcus senilis of the cornea, likewise represent deposits of cholesterol esters. J. Müller reported an unusual case of nephritis, with chylous hydrothorax, and the above-mentioned opacity of the plasma which cannot be cleared by the centrifuge or fat solvents or stained with fat stains. Here the blood contained over 3 per cent. total fat, and 0.6 to 0.8 per cent. each of lecithin and cholesterol. Cholesteremia and lipemia are present with icterus, gall-stones, and liver disease, as shown by Klinkert and Beumer and Bürger. Cholesterol is deposited in the xanthoma formations and the cholesterol content of the bile is said to be increased. Lipemia has also been reported in pneumonia, heart disease, dyspnea, syphilis, and esophageal cancer, some of these cases being perhaps mere alimentary or inanition lipemia. The lipemia in a number of other conditions named by Fischer is doubtful. Experimentally, slight lipemia occurs in poisoning with phosphorus, phloridzin, and other drugs causing fatty degeneration, and during and after narcosis, though the phenomenon is inconstant (Murlin and Riche, Bloor, Lattes and others). Fat emulsions injected intravenously are rather promptly disposed of by the liver and other tissues, and lipemia is resisted. Bloor's highest figure after such injections is 1.5 per cent. blood-fat. It is well known that intoxication with fatty acids has been suspected in the etiology of some clinical anemias. The analyses of Freund and Obermayer, Erben, and Beumer and Bürger show lipemia absent in pernicious anemia and some cases of leukemia, and in other cases of leukemia a slight lipemia up to 0.7 per cent. Bloor gives similar findings in pernicious anemia, with the suggestion that the low cholesterol values may be significant in view of the protective action of cholesterol against hemolytic agents. Boggs and Morris observed lipemia in a man with anemia secondary to hemorrhoids. They compared it with lipemia which they discovered in rabbits made anemic by re-

peated bleedings; they found no lipemia in cases of equal anemia produced by pyrodin poisoning. In the anemia of phenylhydrazin poisoning, Sakai described lipemia of approximately 1 to 3 per cent.; and as observed by Underhill, fatty liver and hypoglycemia accompany such poisoning. When the anemia was produced by bleeding, Sakai found values almost up to 6 per cent. blood-fat, with proportional increase of lecithin and cholesterol. These are the highest figures ever reported for non-diabetic lipemia.

We come now to the principal metabolic experiment which nature has performed for us, namely, diabetes. Just as there is no other way of producing hyperglycemia equal in intensity and duration to that of diabetes, so also the lipemia present in some cases of diabetes is beyond parallel in any other clinical or experimental condition. In blood taken from a thirteen-year-old diabetic child three days before death in coma, Frugoni and Marchetti reported a total ether extract of 27 per cent., and in blood from the same patient at autopsy 34 per cent. The highest figures fully accepted by German authors are 19.7 per cent. by Neisser and Derlin and 18.13 per cent. by B. Fischer. For comparison it is interesting to note that the highest known value for fat in thoracic duct chyle, by Zawilsky with maximal fat feeding in dogs, was only 14.6 per cent. Sakai reckoned that the blood in Fischer's case contained over 700 grams of fat. There are numerous reports of all grades of lipemia below this. Imrie has described one of the most recent cases, with over 14 per cent. fat in the blood. The most comprehensive chemical study has been made by Bloor, who gives complete analyses in thirty-six of Joslin's patients. He found the blood-fat normal or even subnormal in mild cases, but always increased in severe cases, ranging up to twice the normal. Two untreated cases showed the typical excessive lipemia, one with 2.9 per cent. and the other with 11.2 per cent. of fat in the plasma. Such lipemic blood looks like cocoa, and the plasma like cream. Tyson's 1881 text-book and Joslin's 1916 text-book both devote the frontispiece to lipemia, the latter showing the appearance of the blood and plasma, the former depicting the fundus of the eye, for the con-

dition is so marked that it can be recognized by intraocular examination. Normal urine contains a trace of fat, and this is increased in lipemia, Frugoni and Marchetti's case showing 0.088 per cent., Imrie's case 0.1 per cent., and Neisser and Derlin's case the unusual figure of 0.8 per cent. urinary fat.

For closer understanding, inquiry may be made first as to the nature of the fat circulating in such excess in these cases. Analyses from Fischer to Bloor show that the great mass of it is neutral fat. Klemperer and Umber's claim that both lecithin and cholesterol are increased out of proportion to the neutral fat and their suggestion of the name lipoidemia instead of lipemia have been overthrown by more recent work. The lipoids are increased, but the higher the lipemia the greater is the predominance of the true fat. Imrie agrees with some earlier authors in finding lecithin relatively low in diabetic lipemia. Bloor has shown that lecithin varies somewhat in parellel with the total fatty acids until marked lipemia is reached, then falls markedly behind in relation to both these and cholesterol. The striking increase in cholesterol has been noted by authors from Fischer onward. In Imrie's case the blood contained 1.5 per cent. cholesterol. Bloor's case with 2.9 per cent. lipemia had 0.5 per cent. cholesterol, and his case with 11.2 per cent. lipemia had 1.26 per cent. cholesterol. There is evidently a remarkable activity of cholesterol metabolism. The liver is generally bright yellow, but Fischer remarked that the liver cells did not contain large fat-drops as in ordinary fatty livers. The Kupffer cells like the endothelia elsewhere are stuffed with fat, and Kawamura found that this fat consists not merely of glycerides but cholesterol esters, which he claims the Kupffer cells normally refuse to take up. Jastrowitz undertook to study lipoid infiltrations in the fatty livers after various poisons. Beumer and Bürger described a case of diabetes in which an oat-cure cleared up the existing lipemia, but the cholesterol persisted at three times the normal figure. Klinkert states that the white spots in diabetic retinitis represent accumulations of cholesterol esters which may clear up considerably under treatment. The apparent thickening of the vessels of the fundus of the eye in lipemia is due to the opacity

of the plasma and also to the cholesterol ester infiltration of their walls. Xanthomata are an expression of hypercholesteremia in diabetes as in other conditions, though other factors must be concerned. Von Noorden saw them clear up under treatment and return with aggravation of the diabetes. Bacmeister in one case furnished rather doubtful evidence that the cholesterol excretion in the bile may be markedly increased in diabetes. Of other compounds it may be noted that Imrie reported 0.38 per cent. of fatty acids present in the blood as soaps. Aside from the anemia-producing effects, fatty acids and their soaps are highly toxic, Munk finding that 0.11 to 0.13 gram oleic acid as soap injected intravenously in thirty to forty-five minutes suffices to kill rabbits by heart-block. Lipemic patients show no more anemia or intoxication than other diabetics, so it would seem either the findings of high percentages of circulating soap are mistaken or other substances present must protect against its poisonous action. In survey, therefore, it may be said that diabetic lipemia is characterized by an increase of lecithin, which becomes relatively deficient as the lipemia becomes excessive; but comparison with other forms of lipemia is difficult, for it seems probable that if alimentary or any pathological lipemia could be raised as high as diabetic lipemia the relative deficiency of lecithin might be similar. Analyses on stuffed geese would be interesting. Diabetic lipemia is also characterized by a much greater increase of cholesterol, almost parallel with the fat, in excess of anything ever observed outside of diabetes, and in direct contrast to what occurs in alimentary lipemia.

Another contrast is seen in the corpuscles, for instead of the increase which occurs in alimentary lipemia, their fat content amid the tremendous lipemia of diabetes remains normal. Bloor finds the same to be true in other forms of pathological lipemia. The entire chemical picture is interpreted by Bloor as follows. The component which becomes more predominant as the lipemia increases is the true fat, which is the inert form of fat, and its accumulation indicates that the fat is not being properly prepared for assimilation. Likewise the relative deficiency of lecithin and the lack of fat in the corpuscles indicate that the

corpuscles are not performing their function of transforming fat into lecithin, as in the earlier phase of assimilation. The high cholesterol figures are taken to mean that a later stage of the process is represented in this lipemia and that the cholesterol mechanism has not failed. Beumer and Bürger concluded that a considerable part of the cholesterol is free and not in esters, and in Imrie's case practically the whole of the cholesterol was found to be free. This fact might be significant if generally confirmed. Throughout it must be remembered that the entire subject is in the stage of hypotheses, but they are interesting as such and represent a real beginning in attacking the problem.

A second point for inquiry is the source of the fat in lipemia. In alimentary lipemia it is sufficiently obvious that the fat is derived from the food, but the lecithin, aside from what may come from the food, must be supplied by the body. In the various forms of pathological lipemia, lecithin and cholesterol must presumably be supplied by the body; this was certainly true in Müller's case of nephritic lipemia, in which the patient had been on lipoid-poor diet for months. In the anemic lipemia of rabbits, Boggs and Morris showed that the tendency to alimentary lipemia was increased, but yet the essential source of the blood-fat was the body-fat, for the lipemia developed on a diet of bread and grass, the animals rapidly lost weight, and in extreme emaciation the lipemia ceased. Because of the high lipoid content in diabetic lipemia, Klemperer and Umber concluded that the condition represents an increased breakdown of body cells, since only these could furnish so much lecithin and cholesterol. This explanation seems foolish when applied to a lipemia of 10 to 20 per cent. Several authors have followed the hypothesis that the lipemia is derived from the body fat. Magnus-Levy has upheld the opposite view that the blood-fat is derived from the food, that the fat is taken up from the intestine and poured into the blood as usual, but there is some obstacle to its leaving the blood, either a physico-chemical difference in the fat itself or a change in the cells or in the capillary walls; and that the huge quantities sometimes found in the blood may result from slow accumulation. Neisser and Derlin's patient with 19.7 per cent. blood-fat had very little

body fat. They compared the iodin and Reichert-Meissl numbers of the fats in the food, chyle, blood, and several tissues and concluded that the blood-fat comes from the food. Imrie considered that the 300 grams or more of fat in the blood of his patient was too much to be derived from the food; the iodin number of 73 for the fatty acids of the blood compared well with 68 for the adipose tissue, but differed widely from that found in liver, heart, and kidney; he therefore concluded that the lipemia represents mobilization of connective-tissue fat. Bloor observed extreme lipemia only in two patients who had been eating excessive amounts of fat, while severe cases under treatment with restriction of fat as well as other foods seemed to show the tendency but the figures were moderate. Accordingly, he considered that the lipemia is derived from the blood and is due to ingestion of fat beyond the capacity of a weakened assimilative function. It is to be regretted that very few fat determinations have yet been carried out on our patients at the Rockefeller Institute Hospital. The gross observations agree with the experience of Bloor and Joslin that even the most creamy plasma clears up under treatment. One extremely emaciated man showed a diminishing but still opaque lipemia through six days of fasting, which disappeared only gradually in the subsequent treatment. The exit of fat from the circulation must therefore be very slow in some cases. It should be considered a therapeutic duty to clear up a pathologic lipemia.

Dogs are subject to diabetic lipemia, as shown by an observation of Gerhardt mentioned by Naunyn, of 12.3 per cent. blood-fat in a dog with spontaneous diabetes and pancreas necrosis, which is the highest lipemia ever recorded in a dog. It is impossible for a dog to have more severe diabetes than that following total pancreatectomy, but the plasma generally is almost or quite clear. In an exceptional instance Seo observed lipemia of 2.4 per cent., with increase in lecithin and cholesterol. It is possible that Seo's dog was fat and that Gerhardt's dog had been eating fat. The facts perhaps indicate that the lipemia does not represent mobilization of tissue fat by the intense metabolic disturbance, unless to some extent in a fat-rich animal, but that on the

TABLE I.—DOG 396 (PARTIALLY DEPANCREATIZED).

Date, 1916.	Blood.‡ Plasma sugar, %	Hb., %	CO_2, capacity, Vol %	Total acetone,* mgm. per 100 c.c.	Lipemia, qual.	Total fat, plasma %	Urine. Volume, c.c.	Total acetone,* mgm.	Sugar, gm.	Total nitrogen, gm.	Ammonia nitrogen, gm.	N : NH_3-N ratio.	D : N ratio.	Weight, kg.	Diet.	Remarks.
Aug. 16–17	0.465	102	..	..	0	0.509	1313	761.6	50.55	24.10	1.97	12.25	2.10	..	1000 gm. lung.	
17–18	0.327	96	57.6	..	0	0.512	1358	614.8	59.70	22.80	...	..	2.62	11.16		
18–19	0.400	85	42.4	..	0	0.516	1430	344.9	58.40	25.40	2.58	9.85	2.30	10.90		
19–20	..	..	..	..	..	..	1290	443.8	71.50	22.60	1.74	13.00	3.16	..		
20–21	0.356	104	36.2	33.9	0	0.586	1220	873.5	67.20	23.80	1.83	13.00	2.82	10.80		
21–22	0.384	103	..	12.5	++++	1.752	410†	111.4	19.20	5.77	0.45	12.80	3.33	10.70	400 gm. lung; 200 gm. suet.	Eats entire diet taking suet first.
22–23	0.324	98	46.2	29.0	++++++	1.600	804	405.2	24.92	11.58	1.36	8.52	2.15	10.82		
23–24	..	..	..	..	..	..	985	425.5	26.35	11.20	1.88	5.95	2.35	10.86		
24–25	0.285	59	48.1	70.8	++++++	0.835	860	359.9	31.85	10.50	1.37	7.66	3.03	10.70		
25–26	..	..	..	..	..	..	915	786.9	19.08	11.30	1.10	10.30	1.68	10.80		
26–27	..	..	..	..	..	..	782	297.1	26.00	10.95	3.98	2.75	2.38	..		
27–28	0.268	39	32.8	97.8	+	0.445	1018	260.6	24.20	10.15	..	..	2.38	10.75		Growing weak; fatty diarrhea.
28–29	0.385	64	30.9	57.5	0	0.533	922	191.8	26.40	8.64	1.37	6.30	3.06	10.25		Diet forced; vomited; 5 gm. sod. bicarbonate.
29–30	..	..	..	...	..	..	720	123.8	30.00	10.30	2.74	3.76	2.91	10.20		
30–31	0.294	45	38.6	67.0	0	0.365	1020	..	24.40	9.50	4.40	2.16	2.57	10.17		
31–Sept. 1	..	..	..	..	..	..	938	..	24.10	6.60	3.00	2.20	3.65	..		Moribund; killed.

* Total acetone bodies as acetone. † Incomplete specimen. ‡ Blood drawn twenty-four hours after feeding.

TABLE II.—DOG 280 (SEVERE DIABETES).

Date.	Blood. Plasma sugar, %	Hb., %	CO_2, capacity, Vol %	Lipemia, qual.	Urine. Total nitrogen, gm.	Ammonia nitrogen, gm.	N : NH_3-N ratio.	Sugar, gm.	D : N ratio.	Diacetic acid.	Remarks.
Aug. 11–12	..	..	..	..	3.07	0.930	3.30	6.35	2.07	+++++	Fasting.
12–13	0.345	70	24.2	+++++	15.21	3.66	4.16	37.92	2.50	+++++	
13–14	0.435	70	29.0	++++++	7.19	0.50	14.40	7.66	1.06	+++++	Bicarbonate. Death.

TABLE III.—DOG 345 (PARTIALLY DEPANCREATIZED).

Date.	Weight.	Blood.‡						Urine.†								Diet. Remarks.
		Plasma sugar, %	Hb. %	CO_2, capacity, Vol %	Acetone, qual.	Lipemia, qual.	Total fat, plasma %	Volume, c.c.	Acetone, qual.	Total acetone,* mgm.	Total nitrogen, gm.	Ammonia nitrogen, gm.	N : NH_3-N, ratio.	Dextrose, gm.	D : N ratio.	
June 8	11.25	0.232	112	..	0	0	0.92	..	..	..	..	..	..	..	..	
9	..	..	..	..	0	++++	..	778	+++	43.6	6.900	..	..	70.79	..	
10	..	0.276	109	..	0	++++	3.88	880	+++	80.1	6.432	0.730	8.80	73.30	..	
11	..	..	..	..	..	..	..	835	++++	48.4	3.932	..	..	39.83	..	
12	10.5	0.228	110	..	?	0	2.42	562	+++++	25.9	3.844	0.612	6.26	43.27	..	200 gm. lung; 100 gm. bread; 100 gm. suet.
13	..	..	..	..	..	..	..	764	+++++	10.7	4.769	0.609	7.85	34.63	..	
14	..	0.213	108	50.0	+	+++++++	7.12	1270	..	58.4	5.092	0.531	9.58	65.65	..	
15	10.75	0.333	112	57.6	+	++++++	6.00	940	++	..	5.038	0.394	12.80	64.95	..	
16	..	0.313	106	..	..	+++++	2.27	985	..	.	9.600	0.610	15.72	61.56	..	
17	..	..	..	..	..	..	..	990	..	..	6.672	..	..	61.87	..	
18	..	..	..	..	..	..	..	732	..	..	4.721	0.490	9.65	48.82	..	
19	10.85	..	..	..	..	..	..	1180	..	94.0	3.327	..	..	49.08	..	
20	..	..	..	..	..	..	..	598	..	35.9	2.738	0.203	13.42	44.35	..	200 gm. lung; 100 gm, bread; 150 gm. suet.
21	..	..	..	..	..	..	..	1370	..	..	5.918	0.479	12.38	59.59	..	
22	..	0.244	90	..	++++	+++++++	3.16	1810	+++++++	1042.6	5.249	0.796	6.60	78.73	..	
23	..	..	..	..	..	..	..	1270	+++++++	812.8	6.731	0.792	8.50	68.58	..	Unwell.
24	..	..	..	..	..	..	..	2090	..	1097.3	3.762	0.501	7.55	58.30	..	
25	..	..	..	..	..	..	..	915	..	657.8	6.405	0.602	10.66	67.71	..	
26	..	0.238	93	38.5	..	+++	3.79	590	+++++++	367.0	4.838	0.489	9.88	40.45	..	100 gm. lung; 100 gm. bread; 100 gm. suet.
27	..	..	..	..	..	..	..	804	..	313.6	3.939	0.426	9.26	64.32	..	
28	10.82	..	..	..	..	..	..	1285	..	..	4.011	0.552	7 26	75 68	..	
29	..	..	..	..	..	..	..	620	..	282.7	2.852	0.303	9.40	47.74	..	
30	10.65	0.213	96	40.4	..	+++	..	690	+++++++	298.1	3.008	0.324	9.26	43 20	..	Unwell.
July 1	..	0.222	97	..	++	+++	..	396	..	182.1	7.680	0.760	10.10	30.50	3.97	
2	..	..	..	..	..	..	..	608	..	307.8	12.800	1.137	11.21	35.90	2.80	600 gm. lung. Improving.
3	..	..	..	..	..	..	..	332	+++++++	127.2	8.370	0.713	11.78	23.90	2.86	
4	..	0.270	109	34.2	++	+++	1.53	636	..	359.3	16.250	0.850	19.11	30.60	1.88	
5	..	0.256	94	43.9	..	..	0.78	405	..	..	9.450	0.940	10.10	31.20	3.30	
6	10.3	0.323	90	36.1	..	0	0.90	830	+++++	192.6	16.600	1.930	8.60	51.90	..	
7	..	0.250	86	39.1	+	0	..	728	+++++	180.2	9.770	..	..	47.10	..	600 gm. lung; 100 gm. bread. Good condition.
8	..	0.357	86	47.7	..	0	..	1585	+++++	518.3	14.300	1.100	13.00	106.20	..	
9	..	..	..	..	..	..	..	1600	++++	488.0	15.400	..	..	94.40	..	
10	10.13	0.304	86	46.4	+	0	1.98	2025	++++	559.0	10.400	1.010	10.30	93.10	..	

* Total acetone bodies as acetone. † Not catheterized. ‡ Blood drawn twenty-four hours after feeding.

contrary it mostly represents deficient assimilation of fat, and that depancreatized dogs with their maximal diabetes show little lipemia because in them the deficiency in assimilation is balanced by the deficiency in digestion of fat.

This supposition can be tested in dogs of the type described at the outset, which have severe diabetes along with satisfactory digestive power. It is found that they are in fact subject to diabetic lipemia in its full intensity. These dogs have been fed suet, which is not the most easily or rapidly digested form of fat; but approximate determinations in our first case indicated lipemia rivalling that of Gerhardt. The tables show some values incidentally observed in connection with other work. Here is a sample of plasma from a child entering the Institute Hospital in coma. It contains 11 per cent. fat. The analyses made thus far show wide differences in the curves of fat in the blood of normal, phloridzinized, and diabetic dogs after identical feedings. The minor fluctuations will require further study, but the outstanding feature is the disproportionate increase in the diabetic animal. Granting severe diabetes, the lipemia varies largely with the digestive power. The record of dog 396 (Table I) shows how the lipemia fell as the digestive power failed and the plasma became clear. These figures represent analyses twenty-four hours after feeding. On withdrawing fat from the diet lipemia clears up in from one to several days, according to its intensity, as seen in dog 345 (Table III). A possible exception to this rule may occur in a type of fasting acidosis to be described later. In the terminal state of dog 280 here dipicted (Table II) gross observations gave the impression of an increase of blood-fat up to the development of a marked lipemia on fasting, but the opacity of the plasma was the only index, and this chance for a decisive verdict concerning the possible occasional origin of diabetic lipemia from body fat was lost through inability to carry out the necessary analyses.

The production of diabetic lipemia in dogs is a simple matter, but it opens opportunities. Here we have the means of flooding the body with fat in a manner unparalleled outside of diabetes, of making and unmaking this abnormality easily and quickly.

The first result is the conclusive proof that the lipemia is derived ordinarily from the food-fat. It will be a simple matter to feed a variety of fats and compare with the blood-fat in the different cases; some information concerning fat assimilation may thus be gained, and we may be able to report such experiments later. But the simple fact that the lipemia appears so readily on feeding fat and ceases so promptly on omitting fat suffices to settle the dispute concerning its usual origin.

It is expected to publish later some analyses of the lipoid content of the blood and various organs, but this phase must be omitted at this time. As far as comparison is possible between Seo's analyses of liver tissue in diabetic lipemia and those of Jastrowitz and others of fatty livers produced by various poisons, no indication is offered of any specific chemical character of the organ infiltration in diabetes. The huge amount of circulating fat and the remarkable activity of lipoid metabolism offer other opportunities which we shall not be able to follow up. The unusual quantities of cholesterol that seem to be formed invite a study of the excretion in bile and feces and other features important to those interested in cholesterol metabolism. Various problems lately under investigation by authors such as Aschoff, Landau, Mulon, and Borberg concerning the morphology and the chemistry of fats and the function of the adrenal cortex and other organs in regard to them may perhaps be studied with special advantage in a condition in which the lipoid metabolism is specially disturbed or exaggerated.

The metabolism of matter and energy has never been studied in human patients with extreme lipemia, and to avoid confusion from acidosis or other factors it is desirable to make a series of comparative observations on the same individual in the lipemic and non-lipemic condition, and this can be done most conveniently in animals. All are now familiar with the work in Lusk's laboratory, which has shown that the combusion of any food is increased as the supply of it to the cells is increased. Ingestion of any food increases the total metabolism more or less according to the kind and quantity of the food. Ingestion of fat causes alimentary lipemia and the combustion is preëminently of fat. Bleibtreu's

geese, stuffed with rye, with lipemia up to 6 per cent., are said to have shown respiratory quotients as high as 1.33. Whether the figures are strictly correct or not, it seems evident that values above unity were present, indicating combustion preëminently of carbohydrate and the formation of fat from carbohydrate. This means one of two things: either the carbohydrate or the general plethora inhibited the combustion of fat in spite of 6 per cent. fat in the blood—in which case Lusk's law of summation of stimuli is reversed—or if there was combustion of fat in the remotest degree proportional to the lipemia, the total metabolism or the formation of fat from carbohydrate must have been tremendous. Interesting modifications of this experiment might be made by giving fat-rich instead of carbohydrate-rich diet or by using partially depancreatized geese. It is well known that in diabetic patients or animals alimentary hyperglycemia is more pronounced than normal, but the effect on the respiratory quotient is less, and in severe cases may be absent altogether, and this is one of the best evidences of deficient combustion of carbohydrate in diabetes. The case with fat is different, for diabetic patients and even totally depancreatized dogs always burn fat readily. The distinction is not absolute, for depancreatized birds—chickens, ducks, geese—show intense hyperglycemia with little or no glycosuria and can even receive considerable carbohydrate by feeding or injection and dispose of it somehow. Their kidneys are highly impermeable to sugar, but there is a difference beyond this, for feeding or injection of sugar in severely diabetic dogs with renal impermeability means rapidly fatal hyperglycemia—possibly 2 per cent. blood-sugar. The diabetic birds do not metabolize their carbohydrate normally, for glycogen is deficient and emaciation and death occur. The mammalian kidney is almost impermeable for fat, so that lipemia cannot be checked by excretion; and the presence of acetone bodies indicates some abnormality in fat combustion. But the known abnormality consists merely in incompleteness in the end products; no experiments have ever indicated any difficulty on the part of the diabetic in attacking the fat molecule. Patients and suitable diabetic animals often go along on a certain level of marked hyperglycemia, without glycosuria,

and evidently burning some carbohydrate. They seemingly require a higher ''pressure'' of sugar in the blood in order to accomplish the combustion of sugar. In the moderate hyperlipemia ordinarily present in severely diabetic patients, Bloor saw evidence of a similar need of increased fat ''pressure'' in the blood in order for the cells to burn fat. There is opportunity to test this idea with respiration experiments. In contradiction to the prevalent belief of normal fat assimilation in diabetes, investigation will probably show that a certain level of lipemia does not have equal metabolic influence in non-diabetic and in diabetic lipemic conditions. It will very likely be found that the effect on the gaseous exchange is slower and of less degree in diabetic lipemia, corresponding to the known facts concerning hyperglycemia in the milder cases, so that an alimentary lipemia of 2 or 3 per cent. in a normal animal may represent a greater activity of fat metabolism than much higher blood-fat values in an animal with diabetic lipemia. Also, it may be found that the metabolic effect varies among diabetics in proportion to their susceptibility to lipemia, and conceivably may not be fully normal in any severe diabetic. Studies of this sort will throw light on the ability of the diabetic to attack the fat molecule; they may help to show why fat-feeding seems sometimes neither to strengthen nor build up a patient; they will indicate what significance may be assigned to lipemia in the question of metabolism in diabetes; and by completing the proof that lipemia is due to deficient assimilation rather than increased mobilization of fat (even if increased mobilization sometimes occurs), they may contribute an analogy in support of the dominant belief that the hyperglycemia is primarily due to deficient assimilation rather than increased mobilization of sugar.

Our investigation of lipemia by the use of diabetic dogs to date has dealt chiefly with the problem of the actual cause of it, its relation to other diabetic phenomena and to the internal function of the pancreas, and the information which it may furnish concerning the fundamental diabetic condition. Besides the origin from food-fat, certain other questions can now be definitely answered.

First, the visible fat is not in the abnormal form insoluble in ether. Seo found in his one case that the opacity was cleared by ether, and the same has been our experience.

Second, the power of plasma to hold fat in clear solution is not diminished. In connection with the hypothesis of combined sugar, I formerly suggested a possible analogy with fat, in that lipemia might be due to deficient combination of the fat, and raised the question whether the pancreas supplies anything of importance for this combination. Reicher determined the fat before and after digesting blood with pepsin-hydrochloric acid, and concluded that the latter fraction has no importance. Seo mentioned visible lipemia in only one of his depancreatized dogs, yet the blood-fat in the other instances was from 1 to 1.5 per cent. This might indicate an unusually high power of the plasma to hold invisible fat, and the same possibility seems indicated by some of our experiments, and would not be surprising in view of the high lipoid content. Accordingly, no significant reduction in the simple ability of the blood to "mask" fat has been demonstrated. On the other hand, Bloor's belief is that fat must be combined into lecithin in order to be assimilated, and that a deficiency of this combining function is present in diabetic lipemia. The facts at least suggest that methods used for testing the combination of either fat or sugar should not be too crude.

Third, the relation of fat in plasma and corpuscles is of interest especially in connection with Bloor's hypothesis. Unfortunately most of our determinations so far have had to be limited to the plasma, and only a few analyses of corpuscles have been made. Thus far they indicate low total fat content in the corpuscles. Having controllable experimental conditions, it should be possible to trace any significant alterations from the normal through the mildly lipemic animals to the extreme degree in the severely lipemic animals. This research is in progress, but it is better to omit discussion at present rather than attempt conclusions from insufficient data.

Fourth, lipemia is not due to hyperglycemia. For example, mildly diabetic animals, even with abundant fat in the diet, may

be made to show extreme hyperglycemia without corresponding lipemia.

Fifth, lipemia is not due merely to absence of carbohydrate or loss of sugar from the body. Maximal phloridzin poisoning with feeding of nothing but fat, or the longest possible phloridzination on diet free from carbohydrate and high in fat, has failed to produce in dogs anything resembling diabetic lipemia.

Sixth, lipemia is not due to the presence of acetone bodies. Lipemic patients generally have acidosis, but a case of lipemia without ketonuria was described by Beumer and Bürger. It is well known that many patients with severe acidosis and even coma show clear plasma. Bloor found "no definite relation between high blood lipoids and the occurrence of acetone bodies in the urine." In dogs it can be shown that the acidosis of phloridzin poisoning causes nothing like diabetic lipemia, that diabetic acidosis may occur without lipemia, and that marked lipemia may be present without acidosis. Maximal lipemia probably never exists without acidosis, but this is because acidosis goes with the general severity of the diabetes.

Seventh, lipemia is not due to change in the reaction of the blood. The greatest possible reduction of the carbon dioxide capacity by diabetes, phloridzination, or chronic hydrochloric acid poisoning has produced none of the characteristic lipemia.

Eighth, lipemia is not due solely to removal of pancreatic tissue within the limits mentioned. If enough pancreatic tissue is removed to produce even severe diabetes, but the actual occurrence of diabetes is avoided by diet, the characteristic lipemia does not occur. In some such cases alimentary lipemia certainly persists longer than normal, but this may represent merely a slower digestion of fat owing to the smaller supply of pancreatic juice. If the curve of the lipemia is lower as well as longer, it will indicate such delayed absorption. The characteristic of diabetic lipemia is that it both rises higher and falls more slowly than normal. When the assimilative disturbance is slight it may to some extent be balanced by the delayed absorption, so that the mere prolongation of slight lipemia becomes hard to interpret. These experiments are in progress and a sufficient number are

not yet finished to permit positive conclusions. But it is certain, as stated, that the full diabetic lipemia never occurs in the absence of other symptoms of active diabetes.

Ninth, lipemia is not the result of breaking down of a hypothetical "fat function" by direct overstrain of that function. Here again tedious experiments extending over months have been involved. These experiments have proved that the heaviest and most prolonged fat diets, in normal and partially depancreatized animals, neither increase nor diminish the susceptibility to lipemia. If the conditions are such that the fat-feeding gives rise to glycosuria and acidosis, the lipemia begins to mount up; otherwise not.

Tenth is the question of the relation of lipemia to the severity of the diabetes. Up to the time of the present treatment which restricts fat in the diet, high lipemia has been considered a sign of very bad prognostic import. Bloor found some elevation of blood-fat in all severe cases, normal or subnormal values in mild cases. Beumer and Bürger have reported the only known instance of lipemia in mild diabetes. In dogs of the type described above, it is easy to show that the lipemia depends upon the severity of the diabetes. The partially depancreatized dog, with relatively little tendency to lipemia as long as he is kept free from diabetes, acquires the marked susceptibility without any further operation as soon as severe diabetes is brought on by overfeeding with any kind of food. The most striking experiment is to keep the plasma continuously clear by carbohydrate or protein diet, then suddenly give a meal of fat. High lipemia is present within a few hours and persists for more than twenty-four hours. On continuance of fat diet the lipemia mounts to a point governed by the digestive power. As in human cases, it is then unremitting, and like an old hyperglycemia, varies relatively little with meals. With breakdown of digestion, or on withdrawal of fat from the diet, the lipemia clears up in from one to several days, according to its intensity. The tables already referred to illustrate some of these statements.

This permits discussion of the relation of lipemia to the internal pancreatic function. There may be three possibilities: Is

lipemia due to disorder in some organ or in the general system secondary to the original diabetic disturbance? is it another manifestation of deficiency of the same hormone concerned in carbohydrate metabolism? or does it represent lack of some different internal secretion of the pancreas? There is sound justification for speaking of several internal functions of the pancreas. Diabetes is not a mere glycosuria or inability to assimilate glucose. There are abnormalities in the metabolism of protein, fat, and doubtless of mineral substances, which are primary and cannot be reproduced secondarily by phloridzin or any other means. But it is possible that the various functions in question all belong to one internal secretion, and this unitarian hypothesis is inherently the most attractive one. It is a plausible view that the pancreas supplies something necessary for the synthesis and maintenance of protoplasm, that deficiency of this factor makes nutrition of the cells difficult and disposes to breaking down of their reserves, and that this tendency makes itself felt in regard to all classes of foods, but earliest and most manifestly in regard to the most labile and most easily excreted forms. Only positive evidence could justify a doctrine of plurality of internal secretions of the pancreas. The unitarian standpoint would have theoretical importance, for it might reasonably be inferred that the part played by the single hormone would be similar toward the various classes of foods. Glycosuria and non-assimilation of carbohydrate—lipemia and acidosis—increased protein catabolism, aminosuria, and changes in the creatin-creatinin relation—diabetic edema and other anomalies concerning salts—all afford different lines of approach. If all alike are due to deficiency of a certain action of a single hormone, comparisons will aid in learning what the action of this hormone is, and by following the different trails it may be possible to track the thing home and master the secret of the internal pancreatic function and diabetes.

Between the partially depancreatized animal without diabetes or lipemic tendency, and the same animal after feeding has brought on severe diabetes and susceptibility to lipemia, there is only one known anatomical difference, which consists in exhaustion and degeneration of cells in the islands of Langerhans. The

fact that this alteration and the lipemic tendency come on simultaneously, and are typically produced by pure carbohydrate or protein feeding, proves conclusively that the disorder underlying lipemia is bound up to considerable extent with the other diabetic disturbance and is not entirely independent. Possible evidence for the existence of more than one internal pancreatic secretion might be found in the discovery of Lane and Bensley that the islands of Langerhans consist of two different varieties of cells, filled with granules which stain differentially; these cells are believed to be independent in origin and type and not transitional or derivable one from the other. This histological interpretation is strengthened by Homans' discovery that only the so-called Beta cells ordinarily degenerate in experimental diabetes, while the Alpha cells remain preserved even in advanced stages and show particularly dense granulation. This observation was confirmed in the work at this Institute. But it might still be possible that both types of cells are concerned merely in carbohydrate metabolism. Krumbhaar published the description of an important case of spontaneous diabetes in a dog, in which the pancreas was about twice the normal size and its tissue appeared normal; and microscopic examination showed advanced degeneration of the Beta cells everywhere, along with less extreme but still marked exhaustion of the Alpha cells. Lipemia or acidosis was not found in this animal, but it apparently was not studied on fat diet. Martin has discovered that some of our experimental dogs show this same degeneration of the Alpha cells. He is following up the investigation, but there has not yet been time for enough comparisons to establish the possible significance. When a dog shows a dextrose-nitrogen ratio equal to that following total pancreatectomy, it will be interesting to know whether the Alpha cells are degenerated or not. If they are intact their part in carbohydrate metabolism will become very questionable. Material is available from animals of different species, different ages, different grades of intensity and duration of diabetes, on various diets, in nutritive states ranging from obesity to emaciation, with lipemia and acidosis present or absent, and with other physiological or pathological variations, so that it may be possible to

throw some light on the function of the Alpha cells and the unity or plurality of the internal pancreatic secretion.

The facts concerning human patients must also be considered in this connection, and the question whether the full conditions are reproduced in dogs. Granting that all patients with severe diabetes have some tendency to lipemia, is this tendency equal in all of them? When the majority of cases show fairly clear plasma, and a small minority show lipemia of 10 to 20 per cent., can it be maintained that varying quantities of fat in the diet suffice fully to explain such differences? If there is another cause for the discrepancy, does this cause consist in some additional pancreatic disturbance, or in a breakdown in some other organ or in the general system? It is unfortunate that accurate observations covering this point have not been made; but probably most physicians who treat diabetes will have the decided impression that individual variations exist, and that the majority even of severe cases on heavy fat diet are not subject to the most intense lipemia. Though it is difficult to gauge the true severity of diabetes, possibly Beumer and Bürger's patient above mentioned manifested a special susceptibility to lipemia in the presence of only mild diabetes. Several of the patients at the Institute have shown intense lipemia, and it is not evident that their condition was more severe or that they had eaten more fat than some others without lipemia. Certain patients under treatment were tested with heavy fat diets for other purposes and remained free from lipemia. On the other hand, a very few incidental observations seem to indicate that when there has been heavy lipemia, and when it and the glycosuria have been recently cleared up, a meal of fat may cause the plasma to remain turbid for more than twelve hours. The suggestiveness of these chance observations is strengthened by the experience with dogs, in which such a phenomenon certainly occurs. It may prove worth while to investigate whether patients react differently to such a test and whether it signifies a specific weakness of fat assimilation.

Certain observations seem to indicate that in dogs the tendency to lipemia may vary independently of the other diabetic symptoms, and that the governing conditions are at least in part

experimentally controllable. The work in progress must be carried further before it will be possible to decide positively concerning such observations or interpret their significance for the theory of diabetes. The gist of the matter to date is that diabetic lipemia has been reproduced in dogs, and there are hopes that the possible varying grades of susceptibility shown by human patients may be experimentally imitated.

II. ACIDOSIS

The second subject for discussion in connection with the rôle of fat in diabetes is acidosis. Here the primary requirement for clearness is a definition, and the one adopted may be said to rest on three bases.

The first of these is origin and general usage. The pioneer workers of the Naunyn school grasped this problem broadly and deeply; they did the principal work that has been done, and they marked out the fundamental lines which all subsequent research has followed. Hallervorden recognized the significance of the increased ammonia. Stadelmann attributed coma to acid and suggested alkali therapy; it is noteworthy that he used in this connection the term acid intoxication, not acidosis. Minkowski perceived the presence and meaning of the diminished carbon dioxide content of the venous blood. Magnus-Levy determined the balance of acids and bases in the urine. But Naunyn introduced the term acidosis, and said, "With this word I designate the formation of β-oxybutyric acid in metabolism." The name and definition received general adoption, and have been used also by the opponents of the Naunyn school who believe that the intoxication and coma represent something other than a simple shift of reaction. The more recent followers of Naunyn should not pervert his definition, which has been acceptable to both parties; and even if the word must become the exclusive property of either faction, it is not for the losers to carry off the nomenclature.

The second basis of definition is that of need and distinctiveness. Diminished alkalinity, increased hydrogen ion concentra-

tion, lowering of carbon dioxide, decrease of buffer salts, and (for the symptoms of these changes) acid intoxication—all these terms have definite meanings, and to appropriate the name acidosis for any one of them is merely to create a useless synonym. No other name but acidosis exists for the metabolic process which it denotes. Ketonuria and ketonemia have their accurate place but do not cover the ground. Possibly the word ketosis might be suggested and used for special purposes, but the change of established usage would be difficult and seems unnecessary. It may be urged that there are states of increase of other acids, lactic, phosphoric, etc. If desired it may be feasible to include these under a broad interpretation of acidosis, and to distinguish them when necessary from acetone body or diabetic acidosis. But the latter is the original and most important type, and the name acidosis belongs preëminently to it.

The third ground for the definition is its fundamental significance. Here may be seen the sound judgment of Naunyn in defining on the basis of metabolism, not of reaction. A definition must be qualitative not quantitative. Criteria of reaction vary with the tests; the finer methods of today reveal changes not formerly perceptible, and future technic may give truer appreciation of the physiological balance in the blood or may follow changes into the cells. But the metabolic disturbance in question is continuous and must be regarded as a unit. It is recognizable at a time when the protective mechanisms of the body are apparently efficient to prevent any abnormality of reaction, and it persists in spite of any dosage of alkali. Furthermore, a comparison with typhoid fever is illustrative. Fever is a prominent feature in typhoid infection and has been embodied in the very name of the disease. Also, simple hyperpyrexia is a possible cause of death, and rightly or wrongly many physicians believe that they benefit patients and even save lives by treating this symptom with cold bathing or other measures. Nevertheless, the proper definition of typhoid fever must be in terms of infection with Bacillus typhosus and not in terms of fever. Similarly, the acid character of the products in acidosis is important and has received recognition in the name of the condition. Simple dis-

placement of reaction may be a cause of intoxication and even death, and clinical improvement and even the saving of life may be achieved temporarily by the mere administration of alkali. But the metabolic disturbance back of it all is the real thing to be defined and comprehended and treated. A slight objection might conceivably be raised on the basis of rare cases of reported coma without acetone bodies. But there is the old-time answer that such cases though occurring in diabetes may not be diabetic coma; and there is no evidence that a definition based on reaction would fit them any better. For these reasons it seems best to retain the definition of acidosis in the original sense of Naunyn—namely, as that state of metabolism of which the presence of abnormal quantities of the acetone bodies is the one known constant characteristic.

This leads to the question of the origin of the acetone bodies. Their appearance was first ascribed to fermentation of carbohydrate, then to breakdown of body protein. More recent experiments with phloridzin and liver perfusions prove the possibility of a partial derivation from the leucin, tyrosin, and phenylalanin of the protein molecule, while the greater portion of the amino-acids form glucose, and the exact status of some of them is uncertain. But the work of Rosenfeld, Hirschfeld, Geelmuyden, Magnus-Levy and others made it apparent that the principal source of the acetone bodies is fat. The disposal of fat up to the point of its leaving the circulation and entering the cells was discussed under lipemia. Aside from storage, its fate in the cells is supposedly combustion proceeding through successive carbon groups, the best accepted chemical view being the beta-oxidation hypothesis of Knoop, according to which butyric and β-oxybutyric acid may be normal intermediary products and excretion of the latter may represent merely imperfect combustion. One molecule of higher fatty acid could thus furnish only one molecule of β-oxybutyric, and Magnus-Levy calculated that the quantity thus available corresponds to the maximum known excretion, but that in some cases this demands a molecule of acetone bodies from practically every molecule of fat burned. Acetone is a secondary and chiefly abnormal product, but there is a question

which of the other bodies is primary. Formerly, diacetic acid was believed to be derived from β-oxybutyric by oxidation, but Maase, Blum, Dakin, and Marriott have brought evidence that the reverse may be true, and that diacetic acid may be the primary product formed from butyric, and β-oxybutyric be derived from it by reduction. The orthodox belief is that all cells, including muscle cells, burn fat directly. Von Noorden is one of the very few who imagine that the muscles can burn only sugar, which the liver forms from fat for their use, and that acetone body production is associated with the formation of sugar from fat. It is a common belief that the acetone bodies are produced largely or chiefly in the liver. In Embden's laboratory, perfused livers have been shown to form acetone, while kidney, lung, and muscle formed none; furthermore, the livers of depancreatized and phloridzinized dogs formed several times as much acetone as those of normal dogs. Also, Fischler and Kossow phloridzinized Eck-fistula dogs and found that these animals, with ligation of the portal vein and drainage of the portal blood directly into the vena cava instead of through the liver, showed less ketonuria than ordinary dogs likewise receiving 1 gram of phloridzin daily; but with the reversed Eck-fistula, that is, with ligation of the vena cava and drainage of its blood together with the portal blood through the liver, the ketonuria was increased above that of the controls. The experimental evidence thus seems strong, but it requires criticism. It would be well if more work were done along the lines of Fischler and Kossow, to learn whether their results are significant or accidental or whether any other conclusion is possible. Too much importance must not be attached to perfusion experiments or to the milligrams of acetone formed. It may well be conceded that liver cells are able to form acetone, also that acetone formation is more active in depancreatized and phloridzinized than in normal animals. But the negative experiments with muscle and other organs do not prove that they are unable to form acetone bodies or that the quantity which they form is small. For example, authors have reported that the liver perfused with glucose forms glycogen, but no one has demonstrated the formation of glycogen when

muscles are thus perfused, and it is certain nevertheless that muscles in the living body form much glycogen. The function of the liver is primarily metabolic; perhaps for this reason it gives more positive results on perfusion than other organs in which the metabolic is subsidiary to other functions. At any rate, the reason for the close scrutiny of these experiments lies in their disagreement with the chemical views of fat metabolism above mentioned. It would seem that only in the tangled field of diabetes could writers put together such doctrines as the predominant production of acetone bodies in the liver and the chemical views of Magnus-Levy and Knoop, with no consciousness of conflict. If there is anything like the excretion of one molecule of acetone bodies corresponding to each molecule of fatty acid burned, and if it be claimed that any large proportion of the acetone bodies arises in the liver, it follows either that the liver is burning this same high proportion of the fat, or else that the muscles are burning part of their fat perfectly while the liver is breaking up individual fatty acid molecules into several molecules of acetone bodies. Von Noorden's hypothesis is at least consistent on this point. But if, according to the accepted belief, cells in general attack the fat molecule directly, then acetone bodies are formed where the combustion occurs. In Woodyatt's metaphor, the engine "smokes" with acetone bodies. And since the great preponderance of combustion is in the muscles, it follows that the predominant formation of acetone bodies is in the muscles. The only escape would be in an improbable assumption that the muscles burn fat to a certain point and that hypothetical products are conveyed from them to the liver to be formed into acetone bodies. The proof for the chemical theories is not absolute, but it seems stronger than that for the origin of acetone bodies in the liver. Therefore, the most probable view at present is that the formation of acetone bodies takes place mainly in the muscles and other organs and only to a minor extent in the liver.

It is impossible in the present space to review the literature of acidosis or even the literature of fat-feeding, which more directly concerns the present topic. It is well known that fasting human beings regularly show ketonuria. It is not generally appreciated

how widely this phenomenon varies even in supposedly normal persons. Waldvogel and Brugsch observed instances in which fasting produced only trivial acetone excretion. Benedict's fasting man, eliminating approximately 2 to 7 grams of acetone bodies daily, may be considered a fair average. The upper extremes are represented in reports by Von Noorden of excretion of 48 grams in three days of fasting by a girl with gastric ulcer, by Bönniger and Mohr of excretion of over 24 grams in one day by a fasting woman, and by Gerhardt and Schlesinger of 40 grams daily in hysterical vomiting. The available store of body fat is one important factor, and Folin and Denis published a recent illustration of marked acidosis with symptoms in fasting obese women. But it is not certain that this is the sole variable, and only a large statistical study could show whether normal persons of similar nutrition have inherently different susceptibilities to acidosis. Ketonuria likewise results from simple carbohydrate abstinence, and fasting ketonuria is increased by protein-fat diet. Protein is considered antiketogenic in normal persons, the glucose-forming amino-acids prevailing over the others. Evidence that it may give rise to ketonuria has been offered by Rosenbloom and Hurtley for diabetic patients and by Kirk for depancreatized dogs. Such an effect is possible through loss of the carbohydrate portion leaving the ketogenetic portion, through a simple stirring up of metabolism and elimination (just as a submaximal D : N ratio in a fasting depancreatized dog may rise to maximal on feeding), and, in human patients, probably through aggravation of the essential diabetic process. In line with this, a high protein diet is inadvisable for the average patient threatened with coma. Fat constitutes the essential dietary cause of ketonuria in normal persons; for example, Landergren and Forssner thus produced excretion of some 40 grams of β-oxybutyric acid. Attempts have been made to establish the quantity of carbohydrate requisite to prevent acidosis, the figures generally being set at 50 to 150 grams. Geelmuyden found that more, perhaps 200 grams, might be necessary to abolish an existing acidosis, also that the quantity required varies with the quantity of fat in the diet. Zeller worked out a law that for prevention

of acidosis one molecule of sugar must burn for each two molecules of fat, which means the ingestion of one part of carbohydrate for four parts of fat. Von Noorden and his followers have emphasized the wide discrepancies between different diabetics as respects the relation between carbohydrate assimilation and acidosis: for example, Mohr's comparison between two patients under similar conditions, one of them excreting less than 1 gram of β-oxybutyric acid and the other over 15 grams, and his records of other patients with abundant ketonuria while assimilating 120 to 150 grams of carbohydrate. But Mohr mentions a similar discrepancy between two non-diabetics, and Forssner excreted 33 grams of β-oxybutyric acid with 40 grams of carbohydrate in his diet. Gigon tabulates the Landergren and Forssner experiments to show that individual idiosyncrasy is as marked among non-diabetics as among diabetics. It is well recognized, as shown in experiments of Reich quoted by Rosenfeld, that an initial ketonuria generally diminishes on continuance of the same diet. This behavior of normal persons is usually shown by diabetics who do well, and Mohr states that obese persons respond similarly. Folin and Denis observed that repeated fasts in obese subjects produce, so to speak, an "immunity" against acidosis, and the same has been noticed a number of times in our diabetic patients. A minority of diabetics develop serious acidosis on fasting, but when a short period of suitable diet, even protein-fat diet, is interposed, no case has yet been encountered in which a second fast was not well borne. On the other hand the Landergren-Forssner experiments give no indication of any such "immunity" to excessive fat diet. The great lack is of normal data. The Eskimos are much talked about but have never been studied. It is really unknown to what extent the normal human organism can accommodate itself to fat combustion or what proportion of protein or carbohydrate is the minimum necessary permanently to prevent acidosis. Some interesting acetone and ammonia figures ought soon to become available from severely diabetic patients who are kept free from glycosuria for long periods on diets low in protein and carbohydrate. The fat tolerance in such patients seems to differ widely. The susceptibility to acidosis

may perhaps also be governed partly by variables such as the age or the level of nutrition, whether high or low. Even under identical conditions the attempt to establish a universal rule on this point promises to be fruitless, for the reason that the widespread belief regarding acidosis as governed solely by a supposed ratio between fat and carbohydrate in combustion is incorrect. The existing evidence against it may be summarized as follows: (1) the seemingly constitutional idiosyncrasies manifested by both diabetic and non-diabetic individuals, shown in the literature; (2) the acidosis in certain infections, intoxications, liver necroses, and in the cyclic vomiting and gastro-intestinal crises studied by Howland and Marriott and others, in which deficiency of carbohydrate seems an inadequate explanation; (3) the acidosis which Taylor produced in himself by an ash-free diet of seventy-odd grams of protein, 120 grams of fat, and 200 grams of sugar. It is well for those who think of acidosis as necessarily due to lack of carbohydrate to bear in mind this well-authenticated case in which it was produced by lack of salt on a diet adequate in protein, moderate in fat, and liberal in carbohydrate. Rumpf and Joslin's idea of the importance of salts for threatened coma may find an analogy here. The fact that salt starvation has not had this effect in other such experiments perhaps adds to the evidence of personal idiosyncrasy. The experiment might bear repetition in subjects presumably disposed to acidosis, as the obese.

Notwithstanding that fat ingestion has been proved to create or increase ketonuria in both normal persons and diabetics, fat has remained the one unrestricted food in diabetes. Even Forssner saw reasons to justify the prevailing treatment, considering that tolerance for fat is acquired, that its addition to protein then increases ketonuria by only a few grams, and that its use is preferable to undernutrition. Naunyn, von Noorden and all others have agreed that fat should be withdrawn only in the presence of threatened coma. The few writers who have advocated occasional restriction of fat have merely favored limiting it to the caloric requirement of a maintenance diet. The more common practice has been to push fat by all possible devices

to the utmost limit of the digestive power, with the idea of building up strength and nutrition. Another support for the fat diet was given in the statement that the heaviest fat feeding only slightly increases the combustion of fat, the surplus being stored. In this connection the question arose why then ketonuria should be increased by fat ingestion, and various authorities inclined to the view that food-fat may somehow behave differently from body fat in metabolism. Murlin and Lusk proved that six hours after taking 75 grams of fat, a dog's heat production may be 30 per cent. above the basal. The protein-sparing power of fat is known to persist in diabetes. Therefore the absolute and relative increase in fat combustion now appears a sufficient explanation of the slight increment of ketonuria following any single fat meal, and the summation of such effects presumably accounts for the results of longer feeding, so that there is at present no evidence of a metabolic distinction between food fat and body fat. The relief of diabetic acidosis by fasting is doubtless due not only to diminished combustion of fat but also to a beneficial effect of undernutrition upon the assimilation of all classes of food. The fact that patients with severe diabetes frequently become almost free from acidosis, under the circumstances which give rise to a very appreciable acidosis in normal or mildly diabetic persons, would not appear so paradoxical if we had adequate information concerning the reactions and accommodations of normal subjects under truly comparable conditions. The diabetics merely demonstrate a reserve power in the human organism which normal persons could doubtless bring forth under an equal stimulus. Typical of the former treatment of diabetes has been the period when the patient was evidently developing this power and becoming able to live on protein-fat diet with little or no ketonuria; then the later period with heavy ketonuria, whether sugar-free on strict diet or glycosuric on mixed diet, and the necessary end in coma. The moral is that the natural or reserve powers of assimilation should be protected in treatment and should not be broken down by overfeeding with fat or any other food. The material for clinical experiments heretofore has comprised either fairly mild cases or severe cases with the usual heavy and fluctuating ketonuria. The

tests under these conditions have failed to reveal the insidious and cumulative injury caused by fat. When severe cases are made free from glycosuria and ketonuria, a material is afforded upon which any careful clinician can convince himself of the harm of excess of fat. Washing butter to remove traces of lower fatty acids while overwhelming the system with fat which must necessarily be katabolized into lower acids is one of the absurd practices of past treatment now abandoned. And finally it is to be noted that severe cases kept alive for months or years on low protein and carbohydrate, with glycosuria and acidosis kept up essentially by fat, are the cases that offer the greatest difficulty for successful treatment or for building up a tolerance for any kind of food.

It is important to extend research on acidosis to species other than man. A really satisfactory reproduction of the human condition is one of the greatest needs, for the very sake of the knowledge of how to produce it, and also for the opening up of a subject which always comes when it is made susceptible to animal experimentation. It is also desirable to study this disorder in species which do not so closely imitate man, because in man certain features are found quite regularly associated, and are generally conceived as belonging together, and it is valuable to learn whether this association is inevitable, and if not, to take such an opportunity to study the individual factors thus separated. There is no known laboratory animal which reacts precisely like man in this respect. Some apes or monkeys may be expected, according to Baer's findings, to show fasting ketonuria; but the large ones are too scarce and expensive, the smaller ones lack stamina, and it is doubtful if any of them can meet the requirements of appetite and digestion. Other species, as a rule, show ketonuria neither on fasting nor on protein-fat diet. A distinction is generally held between carnivorous and other animals, presumably on the assumption that animals accustomed to carbohydrate will have difficulty in burning fat without it, and on a vague generalization of the observations that dogs and cats are less easily subject to acidosis than man. The first thing learned in studying a variety of species is that this distinction

is wholly mythical. There are differences between species but none between classes of animals. Baer observed that herbivora are as immune to fasting ketonuria as the carnivora. He reported ketonuria in a pig on fasting but not on protein-fat diet. A pig which we studied at the Institute proved more resistant to ketonuria than any dog; and persons who may have cherished a secret objection to being classed as the metabolic brothers of the omnivorous pig may be gratified by our experience that no other mammal reacts less like man. On the other hand the typically carnivorous badger shows ketonuria, which in diabetes begins almost simultaneously with the glycosuria. The dog is the best and most human of animals in the laboratory as elsewhere. He talks with his eyes and tail instead of his tongue, and there are some metabolic differences of similar degree. But he has told us so much of what we know about diabetes that it would be important to find a way for him to reveal the one thing on which he has thus far given scanty and unsatisfactory information, namely, diabetic acidosis. There is evidence that the dog stands ready as usual to do his part, and the fault has been with us. Von Noorden and Mohr have tried to make the matter too simple by affirming [1] that if a dog is kept a long time on bread diet so as to accustom him to carbohydrate like man and then changed suddenly to strict meat diet, a heavy ketonuria results. No experiments are cited in support of this assertion, which would seem to be imaginary; at any rate it is untrue, as we have found in a sufficient number of dogs, some of which had lived on carbohydrate for their entire lives. But Neubauer states the observation that very young dogs may show ketonuria on fasting, thus presenting an unusually close similarity to man. Veterinary literature proves that dogs are susceptible not only to spontaneous diabetes but also to the termination in coma. Fasting phloridzinized dogs show heavy ketonuria, and von Mering, Lusk and others described the limp and semi-conscious state which may result. Marriott demonstrated ketonemia in such animals, and we have found high ketonuria and low carbon dioxide in the

[1] Von Noorden, Die Zuckerkrankheit, 1912, p. 137.

terminal condition, which therefore seems truly analogous to diabetic coma. But as usual there are differences between phloridzin poisoning and diabetes. According to Baer a phloridzinized dog shows ketonuria only when there is a negative nitrogen balance. Perhaps this is why Geelmuyden found fat to diminish the ketonuria. None of our experiments have dealt with nitrogen balance sheets, but the impression certainly is that phloridzinized dogs show ketonuria on diets moderate in protein and high in fat, and this is strongly in accord with the probabilities. The most important distinction lies in the effect of carbohydrate and protein. Benedict and Osterberg proved that protein feeding causes a fall of 50 to 90 per cent. in the ketonuria, even though the D : N ratios showed that all sugar formed from protein was quantitatively excreted. Obviously, protein does not work such a transformation in diabetic patients, and these authors correctly concluded that great caution must be used in interpreting the results of acidosis experiments in phloridzinized animals. The great majority of totally depancreatized dogs show only slight ketonuria and no coma or other acidosis symptoms. Sass, using the Loewy-Zuntz titration method, could detect no lowering of alkalinity in their blood. From the large number of depancreatized dogs in the Minkowski clinic, Allard was able to report several dying in coma with considerable ketonuria. Kirk notes some similar deaths in his pancreas-fed dogs, also a higher ketonuria when fat was fed along with pancreas. Apparently the lower D : N ratio of the depancreatized dog suffices to explain the less marked acidosis as compared with the fasting phloridzinized dog, especially in view of the large quantities of protein katabolized. The great objection to totally depancreatized and Sandmeyer dogs is their cachexia and defective digestion.

Having dogs with severe diabetes and satisfactory digestive power, and desiring to produce in them if possible a facsimile of clinical acidosis, it is reasonable to proceed by subjecting them to the same conditions as human patients. To the question whether successful results can thus be obtained in dogs, the experiments now permit answering yes; that acidosis can be regularly produced in dogs not merely in one way but in three ways, of which

gradations and combinations exist, but which are most conveniently described separately, in order to show the complete imitation of the human phenomena.

First we may take the classical treatment of severe diabetes. This has been based upon a too clever caloric conception. The tendency of the diabetic is to emaciate because of deficient assimilation. The superficially smart idea has been to force up his weight by the trick of crowding calories into the diet to replace those lost in the urine, and of supplying these calories in the form of a food of which the body has only slight ability to relieve itself by excretion, namely, fat. The dog is an ideal subject for such a treatment. On the one hand, by reason of his lower D : N ratio, he loses less sugar than the very severe human cases, and he has a natural high resistance to acidosis; on the other hand, he is able to digest and metabolize far more food per kilogram of body weight than any human being. Therefore, it is an interesting experiment to take a suitable dog, free from cachexia, and see what happens when he is forced either to hold or to gain weight in the presence of severe diabetes. He cannot long hold weight on carbohydrate or protein; the one food for the purpose is fat. It is like the old fancy of the irresistible force meeting the immovable body; and in the present instance either digestion or metabolism, no matter how strong, must break down. Sometimes it is the former. In some dogs, and in any dog if the diet is not carefully adjusted, vomiting, diarrhea, and loss of weight prevent a perfect result. The tendency to digestive disturbances is like that of human patients on similar treatment. If digestion and absorption remain adequate, the breaking down of metabolism is manifested by increasing acidosis. There is repugnance to fat and hunger for carbohydrate as in human patients, but the animal's wishes must be disregarded, as has been done in human cases, and the fat given forcibly if necessary. The highest fat diet is the most quickly toxic, but excessive quantities of fat are not required, and both protein and carbohydrate aid digestion and do not interfere with the result so long as fat is continued. In the long run suet is probably the form of fat best liked and digested. Talcum powder is useful in the diet to

control diarrhea. For the size of dogs used, the acidosis diet has sometimes been 150 to 200 grams suet and 200 to 400 grams beef-lung, or 100 to 150 grams suet, 200 grams lung, and 50 to 150 grams bread. Lipemia is present; there is malaise and depression of spirits as in patients with acidosis, and digestive upsets increase. If the animal is well suited for the purpose, if the diet is properly adjusted, and if there is enough day and night watching of all details, it can be shown that dogs thus go into fatal diabetic coma on full mixed diet. Dog 327 was our first and a very typical case. Table IV shows the clinical details of this final period.

Second, we may take the customary treatment of moderate diabetes and illustrate it in dogs. Suppose that suitable operation and overfeeding have produced a condition in which there is marked glycosuria on a kilogram of lung but sugar-freedom on 800 grams lung, along with a fair state of nutrition and entire absence of ketonuria. Now place the dog on 600 to 800 grams lung and 100 to 200 grams suet, according to the classical method. There is no glycosuria, weight is gained, and the condition is splendid for weeks and possibly months. The treatment is highly successful. Closer examination shows the presence of hyperglycemia and slight ketonuria, which are usual in the patients of corresponding type. Glycosuria follows, illustrating the ''spontaneous downward progress'' which the authorities describe. This is cleared up by a few fast-days on the Naunyn plan and the diet is again adjusted; it may now be 400 grams lung and 200 grams suet. The gain in weight continues as before, with hyperglycemia, ketonuria, and subsequent glycosuria. Again the fast-days are used and the protein diminished, so that the diet is perhaps 200 grams lung and 200 grams suet. The same cycle is repeated. Now the dog is in splendid condition and spirits, the coat sleek, the appearance such that he might create a good impression out walking in the park, only he has difficulty in remaining sugar-free on even the protein minimum, and the fat may be pushed higher to maintain nutrition against the repeated fast-days. If the dog has actually been kept fat, a fasting period about this time may diminish the glycosuria or it may remain

TABLE IV.—DOG 327 (PARTIALLY DEPANCREATIZED).

Date. 1916.	Blood.					Urine.							Weight,† kg.	Diet	Remarks.
	Plasma sugar, %	Hb. %	CO_2 capacity. Vol %	Acetone, qual.	Lipemia, qual.	Volume, c.c.	Acetone, qual.	Total acetone,* mgm.	Total nitrogen, gm.	Ammonia nitrogen, gm.	N : NH_3-N, ratio.	Sugar, gm.			
April 15-16	..	..	..	..	..	922	++++++	91.3	9.082	1.259	7.22	57.33	..	200 gm. lung 200 gm. suet 50 gm. bread	Vomiting and diarrhea frequent during this period.
16-17	0.370	106	22.1	+	++++++	406	++++++	144.8	3.059	0.875	3.50	15.63	..	"	
17-18	0.400	108	29.0	++	++++++	530	+++++	129.9	6.201	0.737	8.40	30.81	19.0	"	Rapidly increasing acidosis symptoms.
18-19	..	..	..	..	..	1186	+++++	134.0	10.081	1.257	8.00	66.22	18.4	"	
19-20	0.400	..	18.5	+++	+++++++	1350	..	489.2	11.475	1.633	7.05	56.70	18.2	"	
20-21	0.314	..	23.3	..	+++++++	1480	..	389.2	10.952	1.717	6.36	51.80	..	"	
21-22	0.500	..	21.4	+++	+++++++	811	..	210.7	4.542	..	..	17.84	16.0	...	Coma.

* Total acetone bodies as acetone. † Note precipitous fall.

TABLE V.—DOG 356 (PARTIALLY DEPANCREATIZED).

Date, 1916.	Blood.						Urine.‡									Weight in kg.	Diet.
	Plasma sugar, %	Hb., %	CO_2, capacity. Vol %	Acetone, qual.	Total acetone,* mgm. per 100 c.c.	Lipemia, qual.	Volume, c.c.	Reaction.	Acetone, qual.	Total acetone * mgm.	Sugar, gm.	Total nitrogen, gm.	Ammonia nitrogen, gm.	N : NH_3-N, ratio.		
Aug,																
24–25	0 200	..	..	..	..	..	269	..	+	..	2.60	7.500	0.820	9.15	11.1	400 gm. suet.
25–26	0.124	106	..	..	..	..	160	..	0	..	+	4.110	0.510	8.07	..	300 gm. suet.
26–27	0.111	..	..	..	..	..	246	..	0	..	0	2.100	1.560	1.35	..	200 gm. suet.
27–28	0.099	..	53.8	..	..	..	192	..	0	..	0	2.670	1.130	2.36	11.4	100 gm. lung; 200 gm. suet.
28–29	..	..	..	..	..	..	352	..	0	..	0	4.100	1.640	2.50	..	Fasting.
29–30	..	..	..	..	..	..	382	..	0	113.1	0	3.570	2.100	1.70	..	100 gm. lung; 100 gm. suet.
30–31	..	..	..	..	..	..	172	..	+	..	0	2.510	1.360	1.84	..	100 gm. lung; 200 gm. suet.
31– 1	..	..	..	..	..	..	140	..	0	..	0	1.580	0.782	2.02	11.4	150 gm. lung; 150 gm. suet.
Sept.																
1– 2	..	..	..	..	..	..	176	..	+	..	0	2.410	0.950	2.54	..	150 gm. lung; 250 gm. suet.
2– 3	..	..	..	..	..	..	160	..	0	..	0	4.320	1.320	3.28	..	150 gm. lung; 250 gm. suet.
3– 4	..	..	..	..	..	..	116	..	+	..	0	1.540	1.020	1.51	..	150 gm. suet.
4– 5	..	..	..	..	..	..	295	..	+	64.9	0	1.593	1.180	1.32	11.75	100 gm. lung; 200 gm. suet.
5– 6	..	..	..	..	..	..	140†	..	++	22.4	0	0.975	0.364	2.66		
6– 7	0.095	90	48.5	0	..	0	357	..	+	86.0	0	3.642	1.175	3.08	11.80	Ditto.
7– 8	..	..	..	..	..	..	258	..	+	49.0	0	2.296	0.413	5.55	..	Ditto.
8– 9	0.083	..	57.9	0	..	0	260	..	+	44.2	0	..	1.000	..	..	Ditto; 8 gm. sod. bicarb.
9–10	..	..	..	..	..	..	842	Alkaline	..	101.0	0	6.399	1.263	5.07	..	Ditto; 5 gm. sod. bicarb.
10–11	0 093	86	67.3	0	..	0	378	Alkaline	0	49.1	0	5.640	0.491	11.50	11.81	Ditte; 10 gm. sod. bicarb.
11–12	..	..	..	..	..	..	380	Alkaline	0	38.0	0	1.642	0.144	11.40	..	Ditto.
12–13	..	..	..	..	..	..	904	Neutral	..	99.5	0	0.814	0.181	4.50	..	Ditto; vomited.
13–14	..	..	..	..	..	..	342	Alkaline	+	68.5	0	2.743	1.180	2.32	..	Ditto.
14–15	0.115	105	67.2	?	..	++++++	170	Alkaline	++++	64.8	0	1.578	0.876	1.80	..	Ditto.
15–16	0 121	93	60.5	+	..	++++	360	Alkaline	++++	84.0	0	3.384	1.332	2.56	..	Ditto.
16–17	0.123	..	58.6	..	..	+	438	Alkaline	++++	53.6	0	3.503	1.051	3.34	..	Ditto.
17–18	0 121	96	64.3	0	..	+	412	Acid	..	30.8	0	0.676	0.144	4.68	12.05	Ditto.
18–19	0.128	..	51.9	0	..	+	220	Alkaline	++++	41.8	0	1.786	0.759	2.36	..	Ditto.
19–20	..	..	..	..	..	..	418	Alkaline	++++	79.7	0	5.643	1.672	3.37	..	Ditto.
20–21	0.149	105	..	+	25.5	+++	664	Alkaline	++++	117.4	0	2.523	1.195	2.10	..	Ditto.
21–22	..	..	..	..	..	..	768	Alkaline	+++++	93.7	0	2.611	1.459	1.79	..	Ditto; vomited.
22–23	..	..	..	..	..	..	670	Acid	..	34.4	0	1.983	0.670	2.95	..	Ditto.
23–24	..	..	..	..	..	..	344	Neutral	..	60.0	0	1.238	0.241	5.12	..	Ditto.
24–25	..	..	..	..	..	..	600	Alkaline	++	..	0	3.108	1.440	2.16	..	Ditto.
25–26	0.179	108	59.5	+++	30.5	++++	870	Acid	++++	..	0	2.302	1.657	1.40	12.20	Ditto.
26–27	..	..	..	..	..	..	790	Alkaline	..	213.3	0	2.465	1.422	1.74	..	Ditto.
27–28	..	98	63.3	0	18.9	+	733	Alkaline	..	43.9	0	1.833	1.393	1.32	..	Ditto.
28–29	0.322	86	61.4	+	..	0	400	Alkaline	..	20.8	0	2.080	0.780	2.66	..	200 gm. lung.
29–30	0.400	..	..	0	31.2	0	565	Alkaline	..	26.2	0	5.763	1.441	4.00	..	
30– 1	0.435	89	..	..	35.7	..	550	Alkaline	+	25.3	5.65	4.554	1.953	2.33	..	
Oct																
1– 2	0.250	92	61.4	0	23.2	0	468	Alkaline	+	..	0	2.621	0.772	3.40	..	
2– 3	0.192	95	59.5	0	..	0	636	Alkaline	0	..	0	2.582	0.670	3.85	..	
3– 4	0.175	88	59.5	0	..	0	605	Alkaline	0	..	0	2.009	0.484	4.14	..	Fasting.
4– 5	0.208	82	59.5	0	..	0	828	Alkaline	0	..	0	2.385	0.621	3.84	..	
5– 6	0.235	88	63.3	+	..	0	842	Acid	0	..	0	2.055	0.505	4.07	..	
6– 7	0.169	76	..	Q	..	0	870	..	0	..	0	2.242	0.686	3.25	..	
7– 8	..	..	..	..	..	..	600	Alkaline	0	..	0	..	0.420	..	..	
Nov.																
3– 4	0.200	..	..	..	..	..		..	..	..	..	..	..	..	8.15	200 gm. lung.

‡ Not catheterized. * Total acetone bodies as acetone. † Incomplete specimen.

high. The previously lively and hungry animal begins to show a curious little mournfulness and complete repugnance to food. A day or two later vomiting of clear mucus begins, and the dog drinks and vomits water. The acetone reaction is heavy; the ferric chloride may be heavy or slight. The alkali reserve of the blood falls low, and the complete picture of patients who go into fatal acidosis on fasting is reproduced. Dogs of the type first described are also subject to this result of fasting if they have been kept fat enough, but fattening is easiest in absence of glycosuria. As in human patients, this form of acidosis more resembles the collapse or heart-failure type of Frerichs. The respiration is less typical and consciousness may be retained practically to the end. The outstanding features are the nausea and vomiting and the profound collapse of strength. Table II represents the terminal stage of this condition in dog 280.

Incidentally it may be noted that the cachexia which sometimes causes an apparent suppression of sugar formation in fasting depancreatized dogs is absent in these severely diabetic, partially depancreatized animals, so that their glycosuria and hyperglycemia typically persist almost or quite to the time of death.

The third type of acidosis in dogs is exemplified by diabetic animals kept free from glycosuria by regulated diet, or by those in which the amount of pancreatic tissue removed is not quite sufficient to give rise to diabetes. They are free from acidosis on protein diet or on fasting, but on a carbohydrate-free diet high in fat they sooner or later develop marked ketonuria. The protein ration may be governed by the capacity of the stomach. Probably high protein tends to increase susceptibility to diabetic glycosuria and diminish the tendency to ketonuria. These experiments also may extend over weeks or months, but we have proved upon many dogs that with enough fat in the diet the result is invariable. The qualitative acetone test is heavy but the quantitative output relatively small, generally below 1 gram. An example of this condition is given incidentally in the record of dog 356 (Table V), with potentially severe diabetes. Partially depancreatized non-diabetic dogs on a diet of 150 to 300 grams suet

and perhaps an equal amount of lung may thrive in spite of ketonuria for a longer or shorter time. Ketonuria is apt to be slight. But the final outcome appears in one of two forms. One may be digestive failure and consequent loss of weight and strength, with cessation of ketonuria. In the other form the routine measures against vomiting and diarrhea may succeed, but the end comes with a remarkable spastic and ataxic condition, with terminal weakness, convulsions and death. The absence of ketonuria in adult normal dogs on fasting has been confirmed, and has been found true also on high fat diet extending over several months and still continuing. Tests on puppies are only beginning. One young collie suddenly showed a heavy acetone reaction after two weeks of fasting. High fat diet was immediately given. On the third day of this the dog, which was not known to have been pregnant, aborted. This was some two weeks ago. The acetone reaction with low quantitative values has not ceased, but the dog continues to act well. It is not known whether this idiosyncrasy is present because the dog is young, or because of the collie breed, or because of the pregnancy. Normal controls flourish indefinitely, without ketonuria or with only traces, on the same fat-protein diet which causes acidosis in partially depancreatized dogs. A certain number of normal dogs with a sufficient preponderance of fat over protein in the diet develop ataxia and fatal intoxication seemingly identical with that of partially depancreatized dogs, but ketonuria is generally absent or slight. Digestion is upset in them as in the partially depancreatized animals; accordingly, ketonuria cannot be attributed to fatty indigestion. This state of intoxication will require subsequent mention. In partially depancreatized animals the sugar tolerance is diminished. If the ketonuria be interpreted as a diminished fat tolerance, it affords no evidence concerning the unity or plurality of the pancreatic secretion, and in absence of evidence the presumption remains in favor of the former. These experiments are mainly useful as showing that a species which normally has a high resistance to acidosis is made readily susceptible by partial pancreatectomy, and as thus furnishing conclusive proof that the simple balance between fats and other

foods does not alone govern ketogenesis, but that a specific internal function of the pancreas is at least one of the factors concerned.

Of various points perhaps deserving mention, six features of canine acidosis will be chosen for separate consideration. First, there are some peculiarities of species seen in the clinical picture. A characteristic of human coma is that the cerebral centers are anesthetized while the respiratory center is stimulated. It may be taken as a general rule that dogs lose consciousness less readily than men, and this applies to their diabetic coma. They begin by showing weakness, drunken gait, and dyspnea especially on slight exertion. The symptoms increase until the animal cannot stand, and the Kussmaul breathing may be typical. The corneal reflex is practically never lost, and even attention to surroundings may be preserved almost to the last. It might appear that the motor centers are selectively intoxicated, until observation shows there is apparent absence of pain in the exposure of bloodvessels or other operations, so that the sensory depression is fully equal to that in human coma, in which a knife-cut frequently provokes some response. The low blood-pressure emphasized by Ehrmann and the soft eyeball noted by Riesman and others have not been tested. Diarrhea sometimes occurs with human coma; in dogs it is invariably present and tinged wtih dark blood. It occurs whether the coma is produced by feeding or fasting, by diabetes or by phloridzin. It is therefore of metabolic, not of digestive origin. At autopsy the liver is typically large and fatty, and much fat may be present in the body. There is more or less venous engorgement of the intestine and other viscera, which is the only apparent explanation of the bloody diarrhea, though the bowel contents begin to appear bloody only toward the rectum.

Second, there is the obvious question of the influence of reaction. Toward the end the carbon dioxide capacity of the plasma falls as in human cases, and to a quite similar level. But one advantage of studying the same phenomenon in various species is seen in the fact that in the dog it is easy to maintain normal or supernormal alkalinity of the blood from start to finish, or to raise it suddenly toward the end when it has fallen. In man this is difficult to accomplish even by the highest alkali dosage,

but some clinicians have asserted that continuous alkalinity of the urine has failed to save their patients. A few personal observations, and my conception of the acidosis process, have convinced me that this view is essentially correct. Probably the typical dyspnea and coma never occur in man or dog except with acid intoxication. Probably the upholders of the acid intoxication theory of coma are correct on this point, which is, after all, their main contention. Where they seem to be clearly wrong is in the more important matter of extending this idea to mean that a maintenance of reaction would prevent intoxication. Conceivably the secret of the improvement sometimes produced by bicarbonate may lie in an unloading of toxic substances from the cells rather than in a shifting of the alkali-acid equilibrium *per se*. Aside from a possible, very brief, rise in blood-pressure, sodium bicarbonate intravenously or otherwise brings no visible benefit to a dog dying of acidosis. Keeping the alkaline reserve of the plasma continuously normal by means of it or any form or combination of alkali salts apparently does not prolong the life of a diabetic dog by a single day. The experiments support the conception that acidosis and coma essentially represent an intoxication due to a specific breakdown in metabolism, and that the tendency to alteration of reaction is only an incidental phenomenon.

A third point, closely connected with this, is the ammonia excretion. This was considered the most reliable index of acidosis until the introduction of the tests of alveolar air and blood. Researches beginning with Walter have established that different species vary in their ability to protect themselves against acid intoxication by ammonia formation, and that the quantity of ammonia that can be produced is partly governed by the amount of protein in the diet. Owing particularly to the work of Magnus-Levy and his masterly reviews of the literature, the prevalent tendency is to see in the ammonia solely a reaction to acid poisoning. Therefore, it is well to read the excellent review by Ewing, which gives fairer consideration to other factors possibly concerned. Since the early work of Minkowski, Schütz and others, it has seemed that the liver function is one governing factor, and

Allard, Rolly and others have upheld the importance of this element in diabetic acidosis. The dog experiments promise to contribute information on this point, but they do not yet permit a conclusion. Normal dogs seem regularly to show low ammonia notwithstanding high fat diet. One of our supposedly normal dogs injured its back while out for exercise, with resultant transitory paraplegia and subsequent polyuria. This dog thrives on either carbohydrate or fat, but shows low ammonia on the former and high ammonia on the latter. Partially depancreatized dogs react to fat diet with such a vigorous ammonia production that the urine is sometimes turned alkaline. Dog 356 (Table V) here gives one example. Alkaline urine on protein-fat diet makes any experienced investigator think of cystitis. To exclude this we now for the most part omit all catheterization and test the animals with a change of food or with alkali. When carbohydrate diet or, as with dog 356, sodium bicarbonate causes a prompt fall in the ammonia and rise in the $N:NH_3$ ratio, cystitis is considered improbable. It is not certain that alkali must necessarily reduce ammonia excretion to normal. The ammonia relations shown in dog 356 and others would seem indicative of reaction or over-reaction to acid. Over against these are the relatively low ammonia figures and high ratios in dog 327 (Table IV), notwithstanding the falling alkalinity of the blood and impending death in coma. The essential known difference between the animals is that dog 327 constantly had carbohydrate in the diet. It is possible that the exceptional results are accidental, and as yet we lack the necessary series of experiments to say that they are significant.

The acetone bodies represent a fourth point requiring brief notice. In general, dogs excrete less of them per kilogram of body weight than human patients. It must be remembered that the excretion by human patients is generally not very high except under the influence of alkali therapy, and coma is possible with low ketonuria. The ketonemia seems to be on a par with that of human patients. It is as if the dog's kidney were relatively impermeable to acetone bodies. Also, sodium bicarbonate seems inefficient to sweep them out. A further distinction is that

acetone represents a higher proportion of the total acetone bodies than in severe human cases. There is greater similarity to the milder human cases in this regard. What significance it may have in connection with the natural difference between dog and man respecting acidosis is unknown. Miss Wishart conceived the idea of applying the Rothera acetone test to the blood plasma. The test has proved very useful as a routine in the animal work. It has also given dependable results when applied to human patients here and in Dr. Joslin's clinic in Boston. The significant color range varies from the faintest tinge up to the deepest permanganate color. The proteins or other substances in plasma seem to cause no interference. The reaction appears unsuited for exact quantitative application, but it gives a quick, rough idea whether there is dangerous ketonemia or not and whether a quantitative determination is worth while; and as its results do not always run parallel to the urinary reactions, it would seem that qualitative tests are more valuable in the plasma than in the urine. Preformed acetone is actually trivial in amount, and the substance in blood and urine indicated by the nitroprusside test and commonly referred to as acetone is essentially aceto-acetic acid (Arnold; Embden and Schliep; Folin and Denis; Hurtley; Kennaway). Specific poisoning with acetone bodies has been the principal hypothesis opposed to the acid intoxication theory of acidosis. In denying the latter view we are not necessarily thrown back upon the former. The cause of damage from the metabolic breakdown may or may not be subject to chemical analysis by present known methods. The present work adds to the means for approaching the problem. It is possible that the acetone bodies may some day prove to be an index rather than the cause of the intoxication in acidosis.

A fifth point for mention is the behavior of the kidney, which has recently been studied in human diabetics by McLean and more particularly by Fitz, but which time has not permitted investigating in our animals. Incidental observations show that the usual polyuria of severely diabetic animals is generally diminished on high fat diet, and oliguria is frequent in spite of high sugar percentages in blood and urine. For some reason thirst is lacking,

so the change is not altogether renal. The kidneys are sometimes remarkably impermeable to sugar, after sugar feeding in mild cases but more notably after fat feeding in severe cases, when it is possible to have 0.4 per cent. blood-sugar without glycosuria. An example is furnished by the record of dog 356 (Table V). Neither water nor sodium bicarbonate has been observed to cause edema, but the latter seems not to produce such thirst and diuresis in acidosis animals as in normal ones, and perhaps this is why acetone bodies are not swept out as in the human. Albuminuria is common in acidosis and in simple fat intoxication, but the few observations made have not shown casts. The kidneys of dogs with advanced acidosis have always shown gross and microscopic alterations as far as yet examined, the Armanni vacuolation of certain tubule cells being the most constant. Glycogenic degeneration described by Ehrlich has not always been demonstrable by Best's carmine stain. From the absence of edema after nephrectomy and other known facts, it is doubtful if dogs are suited for accurate imitation of the renal peculiarities of human diabetics, but it seems probable that excessive fat ingestion has directly or indirectly an injurious effect on the kidneys in both patients and dogs, and that this feature is worthy of more study than we have been able to give it.

The sixth and last point for special mention is the general position of fat in the dietary. The early investigations of the total metabolism founded the conception of the caloric requirement and isodynamic equivalents. A protein minimum has been partially worked out. It has been proved that carbohydrate has a slightly greater sparing action than fat, and particularly by Mendel has been established the importance of individual amino acids for nutrition and growth. The need of salts is known, and the so-called vitamines are a recent discovery. This practically sums up the existing knowledge of nutrition. Against the prevalent belief that a starving organism is benefited by whatever food it can obtain, should be raised the question whether this is ever true of any non-protein food taken in quantities approaching the total caloric requirement each day. The question pertains to the benign carbohydrate. Concerning fat there is no question. Fat

unbalanced by adequate quantities of other foods is a poison. It should be recalled that carnivorous animals subsist largely on protein, and though the Eskimo consumes much fat, he also, according to the Krogh report, eats several kilograms of lean meat daily. After a few days of pure fat diet the most voracious cur will starve to death before he will touch it further, and the more strictly carnivorous cat is still less tolerant of fat-rich diet. Against forced feeding the organism protects itself by vomiting, diarrhea, and remarkable cessation of absorption. No form of emulsification or admixture with talcum or other inert substances to give consistency resembling that of the normal ration avails against this toxic action. The small proportion of protein contained in cream or suet gives only partial protection. The same result follows more slowly whenever the proportion of fat to protein or carbohydrate in the diet is too high. The craving of diabetic patients for carbohydrate is often illustrated in such dogs. It should be worth while to determine a law of balance for normal animals. Not only has the diabetic animal a specific sensitiveness to fat, but on low protein ration it must be unable to bear as much fat as an animal on high protein. If the danger of glycosuria prevents increasing the protein, intoxication can be avoided by diminishing the fat. The animal is thinner but safer, hungry instead of nauseated. Diabetic patients have been treated on protein requirement and caloric computations and on general experience of what they will endure. One feature of this experience is that the great majority of severely diabetic patients acquire a repugnance to the prescribed diet and refuse to endure it. By will-power they sometimes endure it for a time. Raulston and Woodyatt's patient adhered for nearly three weeks to a diet of three eggs and 800 c.c. of 16 per cent. cream daily. They employed this as a temporary measure, but such low protein, full calory diets have been the ideal of the best workers under the Naunyn method. The fact is that such a diet will send a diabetic dog into coma, and it is questionable how long normal dogs could tolerate it. It thus appears that patients were right in much of their conduct, and their stealing of carbohydrate was not entirely due to original sin but was rather prompted by physiological

necessity. They live in fair comfort on moderate protein and little or no carbohydrate as long as the fat is kept suitably low. They behave much more rationally toward simple hunger for all classes of foods than they did toward the former excessive craving for carbohydrate. Lack of self-control still claims many victims, but the proportion of patients willing to follow diet faithfully has been increased by reason of the more natural balance of foods in the diet.

III. INFLUENCE ON CARBOHYDRATE UTILIZATION

The third phase of the rôle of fat in diabetes to be considered now is its influence on carbohydrate utilization, on hyperglycemia and glycosuria.

The literature on this subject is scanty. A small number of writers believe it probable that sugar is formed from fat. A somewhat greater number, including Magnus-Levy, admit the possibility. The great majority recognize the occurrence of such a process in plants, but find no evidence of it in animals. Attempts have been made to demonstrate it in animal experiments, but these have failed so completely that they are not worth reviewing; whereas, on the other hand, the non-increase of sugar after fat feeding, the dextrose-nitrogen ratio, and the respiratory quotient, as found by Lusk and others, offer seemingly conclusive proof of the absence of such a transformation in the intense sugar-hunger of phloridzin-poisoning. Although Donkin insisted on skim milk for his milk cure, and that cream spoiled it, the first to assert that fat increases diabetic glycosuria was Lichtheim, whose perfectly correct statement is mentioned with disapproval by Weintraud. Weintraud argued that even if fat should in certain cases cause the excretion of a few grams of extra sugar, the food value of the fat is far greater than this, so there is clear benefit. This has remained the position of the Naunyn school. Lenné, von Noorden and others, who hold extreme views of conversion of fat into sugar, nevertheless use fat to make up a full caloric ration. Von Noorden confesses to prescribing fat perhaps more liberally than anybody else. He and Falta and other pupils mention occasional so-called "fat-sensitive" patients whose glyco-

suria is increased by fat feeding; but this effect is said to follow only the overnutrition produced by excessive fat ingestion, and fat in the quantities practically employed is held not to increase glycosuria, because if it were withheld its place in metabolism would supposedly be filled by body-fat. I have reviewed elsewhere reports of dextrose-nitrogen ratios supposedly proving sugar formation from fat in diabetic patients, but actually proving they were not adequately watched; also the other observations from Griesinger down to Benedict and Joslin and Du Bois demonstrating that no higher dextrose-nitrogen ratios than Lusk's 3.65 value are found in even the severest clinical diabetes; and the respiratory quotients further assure the non-formation of sugar from fat. In view of the general acceptance of these facts, it is natural that the possibility of increase of glycosuria from fat feeding should be generally ignored. If an occasional voice asserts from time to time that fat increases glycosuria, the protest is directed only against fat rations considered excessive, and only the most slight and transitory undernutrition has been countenanced by such authors.

It is likewise natural that the glycosuria attributed to fat should be small, and that the so-called sensitiveness should be apparent in relatively few cases. The effect of fat was above characterized as insidious and cumulative, even with respect to the acetone bodies which are formed from fat; and at least an equally occult influence should be anticipated with respect to sugar, which seemingly is not formed directly from the fat itself. Its action, though not absent in mild cases, is necessarily difficult to demonstrate. The heavy and variable glycosuria of average severe cases and the already maximal output in the extreme cases hopelessly mask the effect of fat, which therefore is only demonstrable rather doubtfully in an intermediate group. Once more the severe cases freed from glycosuria and acidosis afford the best clinical experimental material. Dr. Fitz has been performing some experiments of this type, with unusually complete laboratory observations. One of his protocols is here reproduced (Table VII). The patient, who developed diabetes at the age of fifteen, is now nineteen, and has been under treatment at the Institute

TABLE VI.—DOG 394 (PARTIALLY DEPANCREATIZED).

Date, 1916	Blood.‡ Plasma sugar, %	Blood.‡ Hb., %	Blood.‡ CO_2 capacity, Vol %	Blood.‡ Total acetone,* mgm. per 100 c.c.	Blood.‡ Total fat, plasma, %	Urine.§ Volume, c.c.	Urine.§ Total acetone,* mgm.	Urine.§ Sugar, gm.	Urine.§ Total nitrogen, gm.	Urine.§ Ammonia nitrogen, gm.	Urine.§ N : NH_3-N ratio.	Urine.§ D : N ratio.	Weight, kg.	Diet.	Remarks.
Aug 6- 7	..	..	..	..	..	1731	360.0	48.5	..	..	..	..	..		
7- 8	0.285	82	47.1	83.2	..	1302	248.0	43.4	..	..	..	..	..	100 gm. lung; 100 gm. suet;	
8- 9	0.270	..	51.0	94.5	0.566	1178	..	60.8	5.30	..	..	..	10.1	100 gm. bread	
9-10	0.250	106	51.0	36.4	..	1900	416.0	80.0	7.40	..	..	..	...		
10-11	0.232	92	..	18.5	0.566	800	130.5	52.0	4.71	0.44	10.71	..	..		
11-12	0.204	..	..	44.8	0.583	630	134.9	23.3	9.50	0.85	11.18	2.45	..		
12-13	..	..	..	..	..	3080†	591.0	19.1	8.65	1.23	7.04	2.21	..	400 gm. lung; 100 gm. suet.	
13-14	0.286	103	32.8	50.1	..	1605	596.0	32.1	11.40	1.61	7.08	2.82	.		
14-15	0.364	95	40.4	23.4	0.490	704	182.4	22.8	9.10	0.91	10.00	2.50	10.0		
15-16	0.250	95	31.9	80.4	0.290	646	124.8	19.4	8.30	0.84	9.88	2.34	..		
16-17	0.377	95	31.9	..	2.380	1030	224.9	35.2	9.27	1.40	6.63	..	9.77		
17-18	0.322	..	51.9	41.0	..	1800	195.1	72.0	10.80	1.62	6.68	..	9.70	400 gm. lung; 100 gm. suet;	
18-19	0.365	90	38.5	30.6	1.840	820	288.0	41.0	9.50	0.82	11.58	..	9.80	100 gm. bread	Dark diarrhea.
19-20	..	..	..	..	..	2350†	742.1	47.0	10.10	1.22	8.30	..	..		
20-21	0.276	..	30.9	73.2	0.430	662	119.6	49.2	8.15	0.76	10.72	..	9.76		
21-22	0.263	51	..	41.1	0.765	406	116.7	13.2	..	0.67	..	..	9.50		
22-23	0.295	35	32.8	39.8	0.591	515	226.5	19.5	7.70	0.77	10.00	2.53	9.50		Unwell; entire diet forced.
23-24	..	..	..	..	..	584	163.8	10.7	5.95	0.73	8.15	1.80	9.07		Eats all diet promptly.
24-25	0.214	39	34.7	36.9	0.825	820	316.8	28.7	8.60	0.90	9.55	3.34	9.30	400 gm. lung; 200 gm. suet.	Seriously ill; fed forcibly.
25-26	0.314	26	40.9	29.2	1.090	686	332.2	15.6	6.65	0.82	8.12	2.35	9.16		
26-27	0.237	..	48.1	..	..	680	214.5	14.3	9.85	0.88	11.15	1.45	..		5 gm. sodium bicarbonate.
27-28	0.256	25	60.5	60.3	0.785	390	170.0	7.2	6.00	1.88△	3.19	1.20	8.75		
28-29	0.354	22	54.8	26.2	0.895	675	230.9	14.1	6.75	1.59△	4.25	2.09	8.51	Not fed	Good condition and spirits.

‡ Blood drawn twenty-four hours after feeding. § Catheterized to separate diet periods. * Total acetone bodies as acetone. † Water spilled. △ Cystitis?

TABLE VII.—PATIENT B. D. P.†

Date, 1916.	Blood.					Urine.							Weight, kg.	Diet.▵			
	Urea nitrogen, mgm. per 100 c.c.	Plasma acetone,* mgm. per 100 c.c.	Plasma chloride, %	Sugar, %	Plasma, CO_2 capacity. Vol %	Volume, c.c.	Total nitrogen, gm ‡	Ammonia nitrogen, gm.	N : NH_3-N ratio.	Acetone,* gm.	Chloride, gm.	Sugar, gm.		Protein, gm.	Fat, gm.	Calories.	NaCl, gm.
Sept. 29–30	..	25	0.594	0.204	63.0	4150	4.31	0.54	7.96	1.67	12.85	Trace	37.8	7.4	13.5	150	10
30– 1	..	..	..	..	..	3235	7.14	0.94	7.60	2.56	6.95	Neg.	36.4	33.0	34.0	450	10
Oct. 1– 2	..	..	..	..	..	3380	8.00	0.98	8.16	2.72	3.89	"	36.1	40.0	47.0	605	10
2– 3	..	33	0.606	0.172	65.4	3560	8.00	1.00	8.00	2.43	8.20	"	36.1	40.0	52.0	650	10
3– 4	..	..	..	..	..	3765	9.08	0.94	9.65	1.85	10.00	"	36.1	40.0	57.5	700	10
4– 5	6.9	25	0.593	0.143	66.3	3970	8.65	0.95	9.10	2.01	13.30	"	36.1	40.0	63.5	750	10
5– 6	..	..	..	..	..	3995	7.75	1.00	7.75	2.62	11.80	"	36.0	40.0	68.5	800	10
6– 7	..	24	0.588	0.192	67.4	3360	8.30	1.44	5.76	..	11.30	..	35.6	40.0	73.5	850	10
7– 8	..	..	..	..	..	3595	9.67	0.93	10.40	2.24	7.74	"	35.9	40.0	80.0	900	10
8– 9	..	..	..	..	..	3745	10.70	1.05	10.20	1.63	11.60	"	35.8	40.0	85.1	955	10
9–10	7.0	26	..	0.204	63.0	3555	9.70	0.89	10.90	1.48	11.90	"	35.9	40.0	90.0	1000	10
10–11	..	..	..	..	..	3835	10.25	0.84	12.20	0.96	12.45	"	35.8	40.0	95.0	1050	10
11–12	..	24	0.582	0.182	68.8	3530	8.26	0.84	9.85	1.35	11.12	"	35.5	40.0	100.0	1100	10
12–13	..	..	..	..	..	3290	9.71	0.95	10.21	1.64	9.37	Traces	35.6	40.0	105.0	1150	10
13–14	..	25	0.581	0.200	65.9	3270	7.64	1.18	6.50	2.50	8.82	"	35.5	40.0	110.0	1200	10
14–15	..	..	..	..	..	3465	10.60	1.18	9.00	3.46	10.39	"	35.6	40.0	115.0	1250	10
15–16	..	28	0.588	0.176	61.2	3120	12.25	1.34	9.15	4.40	8.26	1.85	35.4	40.0	120.0	1300	10
16–17	9.9	..	..	..	..	3140	12.20	1.48	8.25	2.86	10.98	21.32	35.6	40.0	120.0	1300	10
17–18	..	..	..	..	..	3330	10.20	1.63	6.25	4.16	8.66	13.32	35.7	40.0	120.0	1300	10
18	8.4	30	0.587	0.221	59.8	..	..	..	..	..	..	..	..	..	Fasting		

† Entire experiment by Dr. Fitz. * Total acetone bodies as acetone. ‡ Note increased nitrogen output as fat is increased.
▵ 300 gm. thrice-boiled vegetables daily not reckoned; also 300 c.c. soup daily, containing 1.5 gm. N., not included in the protein figures.

for two years. She represents one of the cases in which tolerance is built up very slowly and with difficulty, and on account of tenement environment she loses in a few weeks at home as much as is gained in months at the hospital. Her general condition and power of assimilation are therefore known by long experience. The record begins with high blood-sugar produced by a slightly excessive previous diet. The diet throughout contained a fixed quantity of thrice-boiled vegetables, but no other possible source of carbohydrate. The procedure consisted in keeping the protein intake constant and increasing the fat by about 5 grams daily. The peculiarities concerning chlorides and other features will be discussed by Dr. Fitz elsewhere. The ketone and ammonia excretion increased moderately. The blood sugar first fell, then rose, as the fat was increased. The patient felt better on the higher fat. Such well-being is transitory. Glycosuria appeared in traces and increased to 21 grams. High glycosuria and acidosis can be produced by continuing such an experiment, but safety required checking the injury here by a fast-day. It is feasible in selected cases to show that the symptoms subside on simple omission or reduction of fat. This patient is an example of those who can demonstrably live in fair comfort and nutrition for at least several years on low diet almost or absolutely free from carbohydrate, but who would die rather promptly if the traditional building-up process with fat were attempted.

The earliest experiments showing the benefits of continued reduced nutritional level in diabetes were performed on dogs. Notwithstanding the evident clinical results in severe cases, there remain physicians who, conceding that such methods may be useful for such cases, find in their experience that patients feel best on liberal nutrition, and see no harm in allowing plenty of fat in the cases in which no immediate injury is perceptible. Also, there are other clinicians who hold that alleged radical differences between diabetic patients prevent drawing general conclusions or treating according to a unified broad conception, and who emphasize spontaneous fluctuations due to infections or other causes, and who try to draw distinctions between the properties of various kinds of fats, and who maintain the comfortable inter-

pretation that bad results under their methods are due to a progressive downward tendency inherent in the condition itself. By choosing the severest cases obtainable and including the poor and ignorant we have made a high death-rate inevitable. Furthermore, our own management has not been perfect, and we have sometimes given too high diets and committed other mistakes. This record might be pointed to in support of the allegation that while fasting is good for coma, and has in fact been employed by others previously, the end result is the same anyhow, and in deciding between coma and starvation it is better to choose the former and keep the patients as comfortable as possible. In defense we might point to the very high proportion of these patients apparently saved and improving and enjoying even comfort and usefulness in cases and to an extent apparently impossible under any former methods. Certainly the experience of the great majority of specialists and general practitioners has declared favorably for the benefits of the new plan, and it would not be possible for a treatment to receive a more cordial reception by the medical profession. But both the permanent establishment of the practical treatment on a basis not shaken by every wind of doctrine, and the full determination of the theoretical and scientific significance of the changes thus produced in the diabetic condition, require a clear demonstration of the principles at issue in animal experiments, in which all extraneous and accidental factors can be excluded, and facts concerning the rôle of fat in relation to diabetic glycosuria and carbohydrate metabolism can be proved by methods beyond the scope of the personal equation.

The first step in such an investigation is to define the potentialities of the experimental material. The material consists of dogs or other animals with injury of assimilation produced by a surgical resection. They are seemingly free from variations due to heredity or the innate tendencies ascribed to human patients. Their special peculiarities and possibilities must be learned. Glycosuria is more liable to cease spontaneously in cats than in dogs, apparently owing to a slower tendency to degeneration and a greater power of recovery in their exhausted islets. Thiroloix

and Jacob first announced that mildly diabetic dogs may be sent into the severe fatal form by carbohydrate overfeeding, like human patients, but they gave no description of controls, and their observations were apparently not long enough to reveal the final fate of the dogs not fed on carbohydrate. On first coming to the Rockefeller Institute over three years ago, I set apart some of the first dogs for prolonged experiments covering this point. Some were to be subjected to successive removal of small pancreatic fragments for microscopic and other purposes. Others after one initial operation were merely kept on certain diets. Shorter tests under a year in length were performed on other dogs and other species. No animals succumbed to operation, but a number to the environment. The finished series is not as perfect as planned, but is adequate for decisive results. The proportion of the pancreas which must be removed to produce a given grade of diabetes is fairly constant in a given species, though the rare individual exceptions are sometimes marked. There are great differences between species, which are independent of the natural sugar tolerance, diet, pancreatic structure, or any other known factors. The general tendency of the pancreas is to hypertrophy after partial ablation, most markedly in young animals, as Homans stated. Extirpation to any point short of producing diabetes causes no tendency to degeneration of the remnant or to downward progress clinically. When the operation is sufficient for mild diabetes, the food tolerance as ascertained after allowing a few weeks for recovery from trauma will generally hold good for a number of months on suitable diet. There is a tendency to some gain in tolerance, but this is usually easy to break down. It is hard to distinguish the true from a false gain in tolerance, characterized by hyperglycemia without glycosuria. Previous authors have reported this phenomenon after repetitions of adrenalin and in old human diabetes. It is present at a certain stage of overfeeding with sugar, protein, or fat. Part of it may represent renal damage, but a part is probably some reaction connected with the basic nature of diabetes. Within proper limits the animals are very valuable for testing

the effects of all sorts of agencies upon the carbohydrate and other assimilation and for distinguishing between diabetes and accidental glycosuria. The downward progress conclusively shown by feeding beyond the tolerance with carbohydrate or protein has been previously outlined. The experiments of feeding within the apparent tolerance have been only recently coming to completion. Dogs with a limited carbohydrate tolerance, when kept long enough at normal weight with carbohydrate below the limit, or on lean meat only, or on mixed lean and fat, finally show a gradual fall in tolerance, and all but one of the animals of this series have died of diabetes. The remaining one, now on a protein-fat ration, not only has lost all carbohydrate tolerance but shows active diabetes in the form of constant hyperglycemia and ketonuria. There is no reason why diabetes in an Eskimo should not begin in this way, but the ordinary human diet is such that glycosuria precedes ketonuria. The sudden or slow onset of human diabetes can be imitated in dogs. Several interesting examples of apparent onset after traumatism have occurred, in which it is evident that the trauma merely made active a latent diabetes. This does not exclude the possibility that trauma alone may sometimes cause diabetes in man, any more than the absence of any observed association between diabetes and infection in dogs contradicts the demonstrated facts that infections aggravate human diabetes and that patients have been known to acquire diabetes with an infection and recover completely after the infection. The experiments prove that the ingestion of excessive carbohydrate or protein does not create diabetes but merely hastens its active onset. When the underlying tendency is slight enough, indulgence or avoidance of dietary excess may be a deciding factor. These, in conjunction with former experiments, make it seem probable that luxus consumption of carbohydrate, nerve-strain, and other controllable influences may affect the incidence of diabetes by bringing out latent diabetic tendencies in a population.

The investigation of the rôle of fat in relation to glycosuria was undertaken along several lines. The proportion of pancreas

which must be removed to induce diabetes in fat and thin [2] dogs cannot be shown to differ beyond the limits of experimental error and individual variation. The essential experiments consisted in depancreatizing dogs so that severe diabetes came on with or without overfeeding, and proving that the glycosuria could be stopped by fasting and kept absent on low diet at subnormal weight, while addition of fat to the diet brought on gain of weight and consequent glycosuria. These were the experiments upon which the undernutrition treatment was founded. The requirement was to make them conclusive. Accidental factors must be excluded, and it must be established whether the downward progress of such dogs is prevented or merely delayed. One possible method is to compare a sufficient series of undernourished dogs with the well-nourished dogs and learn which live longest and how the tolerance behaves. This was done, and in several undernourished animals a gratifying improvement of protein assimilation was observed, in contrast to the downward tendency in the controls. But the undernourished animals fell victims to laboratory environment, and the attempt had to be repeated. The longest duration was one year, with continued gain in tolerance and strength, which is not without importance. The undernourished state cannot be blamed for causes of death such as rabies, and badly undernourished street curs appear hardy. But in consequence of the accidents, none of these experiments were long enough to found a fully decisive comparison. The ideal procedure would be to keep dogs sugar-free for a period on a given diet, then fatten the same dogs by adding only fat to the diet and show that glycosuria ensues, and then remove or diminish the fat and prove that glycosuria ceases and tolerance is raised. These experiments also were long, and were carried to the point where the obese dogs developed glycosuria. These were the first deaths in coma, which were important in opening up the study of acidosis, but highly inconvenient in the present connection through spoiling the chance of showing that the identical animals

[2] "Thin," in the ordinary sense, opposed to obese. Long fasting or extreme undernutrition makes a decided difference.

could be free from diabetes at reduced weight. On the whole the experiments along these lines were of a difficult and disappointing character, such as one would not wish to go through with again. Large pancreas remnants were requisite, in order that the dogs might retain appetite and digestion for fat. Animals must be chosen voracious enough to take hundreds of grams of sugar to break down their tolerance, and later to consume enough fat with restricted protein to become obese, as most dogs will not do. The preparatory period of sugar-freedom and fixing the tolerance was tedious, and after some months the dog might refuse to eat enough for fattening and have to be used for some other purpose. Few laboratories could be expected to repeat such experiments, and the outcome would still be subject to a personal factor. The results of briefer tests were in apparent contradiction to the longer experiments. Addition of fat to a diet causes a distinct depression of blood-sugar during digestion, confirming Jacobsen's findings in human patients. Dog 386 gives an example; Table VIII shows how the blood-sugar was lower in every instance on protein plus fat than on the same quantity of protein alone. Intravenous glucose injections do not occasion higher glycosuria during alimentary lipemia than during fasting. The reports of Blum and Roubitschek that fat feeding increases adrenalin glycosuria could not be confirmed. The first part of the record of dog 356 (Table V) shows how feeding of only suet diminished the hyperglycemia in an animal with potentially severe diabetes. Even in the severest active diabetes the feeding of pure fat does not increase hyperglycemia or glycosuria. This statement is not altered by the experience that under some circumstances dogs with severe diabetes show a striking increase of blood-sugar after receiving fat; but though this increase may continue for several hours it may begin almost immediately, therefore is obviously not due to anything derived from the fat; and this view was confirmed by finding that an equal amount of kaolin or talcum powder has the same effect. It will be noticed that all the experiments of this order are inimical to the idea of sugar formation directly from fat. In the face of all the uncertainties and contradictions, there was a distinct belief that the long ex-

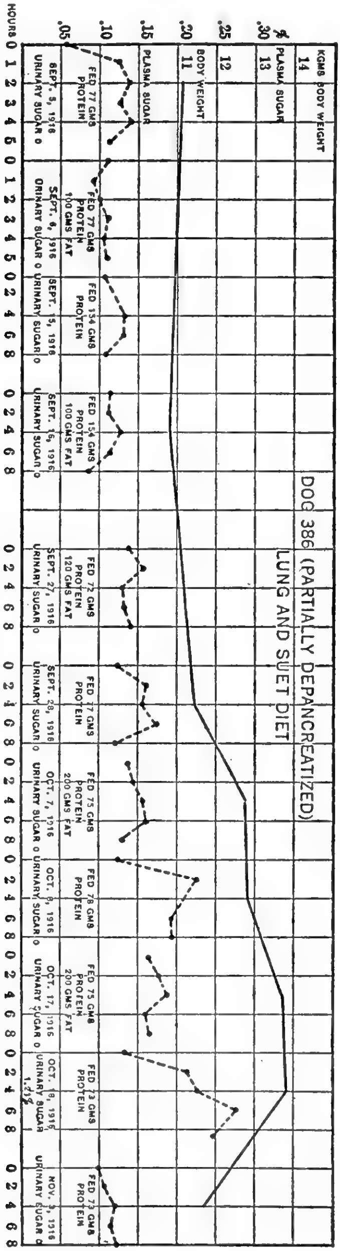
DOG 386 (PARTIALLY DEPANCREATIZED)
LUNG AND SUET DIET
KGMS BODY WEIGHT
% PLASMA SUGAR
BODY WEIGHT
PLASMA SUGAR
HOURS
FED 77 GMS PROTEIN
SEPT. 5, 1916 URINARY SUGAR 0
FED 77 GMS PROTEIN 100 GMS FAT
SEPT. 6, 1916 URINARY SUGAR 0
FED 154 GMS PROTEIN
SEPT. 15, 1916 URINARY SUGAR 0
FED 154 GMS PROTEIN 100 GMS FAT
SEPT. 16, 1916 URINARY SUGAR 0
FED 72 GMS PROTEIN 120 GMS FAT
SEPT. 27, 1916 URINARY SUGAR 0
FED 77 GMS PROTEIN
SEPT. 28, 1916 URINARY SUGAR 0
FED 75 GMS PROTEIN 200 GMS FAT
OCT. 7, 1916 URINARY SUGAR 0
FED 78 GMS PROTEIN
OCT. 8, 1916 URINARY SUGAR 0
FED 75 GMS PROTEIN 200 GMS FAT
OCT. 17, 1916 URINARY SUGAR 0
FED 73 GMS PROTEIN
OCT. 18, 1916 URINARY SUGAR 1.21%
FED 73 GMS PROTEIN
NOV. 3, 1916 URINARY SUGAR 0

TABLE VIII

periments pointed to fat feeding as a potent factor for producing diabetic glycosuria; but the question remained how to make this a positive conclusion instead of a personal impression.

Two changes were introduced. First, a slight increase in the staff permitted following the blood-sugar more satisfactorily. Second, independence of the dog's caprice was gained by forcible feeding when necessary. The appetite of diabetic patients has never been the guide to their diet. Fat has been given to them disguised by all the arts of cookery, and in addition they have been ordered to drink olive oil and otherwise consume fat beyond their desire. It has not been found possible to fool the instincts and senses of dogs when they begin to revolt at fat, but it is feasible to stuff down a ration exceeding what most dogs will continue to eat voluntarily, and if properly planned it is well digested and absorbed. Since the highest fat gives the quickest results, the experiments are thus compressed into a few weeks or months and are convenient to repeat. The numerous controls show the absence of any similar results in dogs not subjected to the fattening process. At the same time successful experiments in which greedy dogs ate everything voluntarily show that the forcible feeding is responsible for no difference; and earlier experiences (above described) prove that the same thing happens when the ration is more moderate, only the time required is longer. Two examples of the recent type of experiments are here shown.

Dog 356 (Table V) underwent operation on July 13, which left a remnant of one-eighth to one-ninth of the pancreas. The tolerance was broken down by feeding, so that it was slightly below one kilogram of lung; that is, on feeding this quantity, glycosuria remained absent until the third day, then was 2.85 per cent. It was cleared up by two days of fasting; then on August 21 a diet lower in protein but higher in calories was given, namely, 400 grams lung and 200 grams suet. This dog had been in barely medium condition at her original weight of 15 kilos. The preparatory stage had reduced her to 11 kilos. The greatly undernourished dog ate all the suet eagerly, and tolerated this excessive ration without glycosuria, until on

August 25 glycosuria of 2.6 grams appeared, with a fasting blood-sugar of 0.2 per cent. Here lung was omitted, and 400, then 300, then 200 grams of suet fed. Under this huge caloric intake, glycosuria ceased and the blood-sugar rapidly fell, giving no sign of sugar formation from fat. The protein was then cut far below the tolerance, and the fat diminished to what the dog could digest regularly. The normal plasma sugars from September 6 to 11 show that this diet of 150 grams lung and 200 grams suet was well within the tolerance at that time. Weight was steadily gained and the blood-sugar rose in parallel, while ketonuria and lipemia developed. The plasma sugar values on September 28 and 29 without glycosuria illustrate the renal impermeability under these conditions. On September 30 glycosuria developed. The diet at this time was changed to 200 grams lung, being approximately the former protein intake with omission of fat; and it was hoped that the course might thus be changed for the better. But the evil that fat does lives after it. A patient threatened with coma may not necessarily clear up if given a protein ration such as he subsequently may come to tolerate well after fasting; and when the injurious effect of fat has been pushed to this extreme point in diabetic dogs they are narrowly saved by using the same treatment as for human patients, although if the program had been changed earlier the simple omission of fat might suffice to reverse the progress. Accordingly, this dog was promptly fasted and there was prompt cessation of glycosuria and ketonuria. Low diet changed the condition so that the weight on November 3 was only 8.15 kilos, and 200 grams lung without fat caused no glycosuria, though the plasma sugar was 0.2 per cent., showing this ration to be still excessive. This is an example of treatment of severe diabetes by marked undernutrition. When the dog was four kilos below medium weight, the attempt to fatten her up to 3 kilos below the weight precipitated a dangerous diabetic outbreak, even though the gain was accomplished by addition of pure fat and there is no indication that sugar is formed from the fat. Not the kind of food given, but precisely the gain in weight above what the assimilative function is able to carry, is the cause of the breakdown in such dogs and

in corresponding human patients. Life is saved by reducing the body mass to what the assimilative power is able to maintain, and if the sparing is adequate, more or less recovery of the function follows in all dogs and in the great majority of human patients. Such an undernourished state as required in this animal, though unpleasant in dog or man, affords the sole means available not only for averting impending death but also for opening the way to a better state. The diabetic has not the choice of a short and merry life versus a long and miserable one. By overtaxing his assimilation he brings on both shortness and misery of existence. These very thin dogs are stronger and happier than the fatter animals with glycosuria and acidosis; and the treatment of fasting and undernutrition for patients has brought not only improvement from the standpoint of laboratory analyses and expectation of life, but also comfort and usefulness and freedom from a multitude of complicating afflictions to a degree never before known.

In other animals as thin as this and with an equally low tolerance, in which the breakdown was produced by carbohydrate or protein, treatment by undernutrition has resulted in steady improvement, as manifested by ability to endure more food and more weight up to a necessary limit. Injury from fat is more lasting and dangerous, presumably because less obvious, so that the harmful process is at work unseen for a considerable time before treatment is applied. This dog 356 finally died in extreme emaciation after a course apparently representing spontaneous downward progress. It furnishes an exact parallel to the type of patients who finally die in spite of treatment, through failing to gain enough assimilative function to support life.

The graphic record of dog 386 (Table VIII) gives a different example of the same principle. The dog came to the laboratory fat at a weight of 15 kilos, and the operation on April 28 left a remnant of only one-twelfth to one-thirteenth of the pancreas. Thus the tendency to diabetes was made more pronounced than in the preceding dog; but in consequence of more careful diet, for a longer period, the actual assimilation and condition were better. The tolerance was approximately 800 grams lung, which

caused slight glycosuria on August 10, when the dog weighed 11.8 kilos, and no glycosuria on September 15, when the weight was about a kilo less. Meanwhile the regular diet was 400 grams lung, which was tolerated for a month without a trace of glycosuria. On September 18 the diet was changed to 350 grams lung and 150 to 250 grams suet, which was continued except for a few test days for just a month. The animal behaved splendidly, enjoyed the diet throughout, and permitted a flawless experiment. The chart shows that at the outset the only indication of severe diabetes in a dog which seemed so beautifully healthy was the fact that protein feeding caused regularly a rise of blood-sugar like that produced by a large amount of carbohydrate normally. This is an interesting peculiarity of such animals. Not only the 400 grams of lung on September 5 and 6 was well borne, but also the 800 grams on September 15 and 16 caused no hyperglycemia greater than 0.13 per cent., thus proving the assimilation. Then the fat was added to the diet and the weight progressively rose. The chart shows how the fasting values for the plasma sugar were constantly within normal limits on the protein diet, also that where the plasma sugar was determined hourly or two-hourly after feeding, the curve after protein plus fat was invariably lower than after the same quantity of protein alone. Nevertheless, as the weight rose the sugar curves also rose. On October 17 the regular protein-fat diet did not suffice for glycosuria, but on the next day the simple omission of the fat sent the plasma sugar so much higher that a glycosuria of 1.21 grams resulted. The attempt to carry this experiment through without fasting was unsuccessful, but on the usual treatment the glycosuria and the ketonuria also present promptly cleared up. On a low diet of protein the weight was brought down to 11.7 kilos, and a test on November 3 showed a fasting plasma-sugar of only 0.1 per cent. which rose only to 0.118 per cent. without glycosuria on the identical protein intake as before. As stated, the operatively produced tendency to diabetes is greater in this dog than in dog 356, but this one represents a case taken before it is so far advanced. This animal can be kept healthy and happy at as high a weight as necessary for either strength

or symmetry. Obesity would bring on diabetes irrespective of the kind of food that produced the obesity.

In these and similar experiments one incidental observation is that the blood-sugar is not an infallible criterion for prognosis. It may be within normal limits both before and after meals on a diet which nevertheless is destined to make trouble later. Also, a very high level of blood-sugar may persist for a considerable time after wiser treatment has changed the direction of progress, so that hyperglycemia does not of itself demonstrate a breaking strain of assimilation or preclude improvement. A more broadly important lesson is that the age-long search of chemists for a magic food which diabetics shall assimilate perfectly is as vain as earlier quests of the holy grail or the fountain of youth or the philosopher's stone. What the diabetic organism is unable to assimilate without restriction is not any particular kind of food, but food as such. From this standpoint all the attempts from the earliest ones with glycerin and lactic acid and levulose down to Rosenfeld's lactone and Grafe's caramel may be judged together and the true reason of their failure appreciated. It is not necessary to conclude that any component of fat is changed into sugar. Although Cremer and Lüthje proved the formation of sugar from glycerin, von Mering first and Lusk more exactly showed that fat feeding does not affect phloridzin glycosuria, and the latter found further that work, which increases fat catabolism, does not alter the D : N ratio, so there is entire lack of evidence that glycerin is split off from fat to form sugar. It may be significant that the experiments and theories of Embden, Neuberg, Dakin, Ringer and Woodyatt, however differing in details, stand in agreement regarding the conception of a merging and equilibrium of chemical products from different sources. It is known that certain substances participating in intermediary metabolism are chemically derivable from either protein, fat, or carbohydrate. Apart from the actual interconversion on a large scale possible between some of these substances, as amino-acids and sugar, it is conceivable that the mere glut of any products is a hindrance to either the anabolism or the katabolism of other products. In such embarrassment of the cells there are certain

substances which most readily escape into the blood and urine; but it must not be concluded from this fact that the diabetic fault of assimilation is limited to sugar (as apparently in phloridzin poisoning) or that the intoxication of acidosis is merely due to the acetone bodies. Wells near the seashore rise and fall with the tide, not because any fresh water is derived from the ocean, but because the drainage of the underground streams is blocked in proportion as the tide is high. Such a comparison may explain the production of diabetic glycosuria by fat for those who do not believe in the derivation of sugar from fat. The primarily ketogenetic and secondarily glycosuric action of fat and the primarily glycosuric and secondarily ketogenetic action of carbohydrate are in accord with this speculation. Janney's investigations of proteins cannot show that any amino-acids are harmless, but may indicate which of them are preferable in cases in which the principal immediate tendency is to glycosuria and in others in which the existing tendency is to acidosis.

The most important fact shown by this series of experiments is that the appearance of spontaneous downward progress observed in human patients can be exactly imitated in dogs. It is possible that further factors, notably occasional infections, may be operative in at least some human cases. It is not positive that the undernourished dogs will be able to live indefinitely. But it is conclusively demonstrated that the attempt at high nutrition, even with fat, produces in these dogs an appearance of spontaneous aggravation of condition as striking as anything witnessed in human patients, and that this result can be prevented at least for periods of years by limiting the total caloric intake and the body mass to correspond to the assimilative function. The experience with diabetic dogs warns unmistakably against efforts to maintain patients on a luxus level of diet or weight. The standard should approach that of Chittenden rather than that of Voit. Restriction of single foods, as carbohydrate or protein, suppresses symptoms temporarily, but lightening the total load upon the weakened assimilative function is the only present means by which it may be hoped actually to halt the diabetic process.

The animal experiments have placed the successful therapeutic

results on something more substantial than an empiric basis, independent of opinions or impressions, and no clinical mishaps, whether due to faulty application of the method or to failure of a defective function to recover by rest, can now shake the principles on which this treatment is founded or justify a return to overfeeding with fat and other mistakes of the past.

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CHEMOTHERAPY IN TUBERCULOSIS *

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AFTER more than ten years of successful activity in its peculiar field, an invitation to deliver a lecture before the Harvey Society is in no small measure a command; accepted in either sense with great pleasure and a feeling of responsibility on my part. My choice of subject was dictated by the request of your secretary that I speak on some topic connected with my own work.

In its previous lectures on the general subject of tuberculosis, this Society has been especially fortunate. As a source of stimulating ideas and points of information of crucial value, the first of these, by Prof. Theobald Smith, is classical. I wish to refer especially to his introductory remarks in order to place the subject with which we are concerned this evening in its proper relation to the whole question of tuberculosis as it can be considered in the laboratory. Professor Smith in 1906 said: "The present day problems in tuberculosis which can be approached by experimental, or at least by laboratory methods, manifest themselves in three different ways:

"1. In the somewhat chaotic condition of opinion concerning the avenues through which tubercle bacilli gain a foothold in the body;

"2. In the wide divergence of opinion concerning the relation of bovine to human tuberculosis; and

"3. In the general trend of studies toward the problem of specific immunity, with especial reference to prevention and treatment."

In the further course of his lecture Dr. Smith presented and critically considered the evidence bearing on the portals of entry

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of the tubercle bacillus. He laid stress on the major significance of the direct infection of the lung and associated lymph nodes by way of the inspired air.

Two years later, Professor Calmette presented a great deal of material bearing on the proposition that the tubercle bacillus gains its entry chiefly through the intestinal mucosa. To-day opinion is perhaps little less chaotic than at the time of these lectures, but the debate has largely subsided due to paucity of competent new evidence.

As the result of exhaustive experimental work in this country and abroad, the relation between bovine and human tuberculosis may be considered to be established.

The third group of problems considered by Dr. Smith has been continuously attacked in the interim. The acomplishments of the period were summarized in able fashion by Dr. Baldwin in this hall two years ago. While immunization has so far led to no practical result in tuberculosis, we should not consider for a moment that the possibilities are exhausted. In the abstract, immunization in some form—active immunization as a preventive measure or combined passive and active immunization during the course of the disease as a therapeutic procedure, most closely simulates nature's own method. If in tuberculosis we have so far achieved no appreciable success along these lines, it is doubtless that success awaits some new technical departure adapted to the exact situation—a development that may come with a fresh point of view derived from the study of some other more or less distant disease or which may more probably come as a matter of direct attack if the search is pursued with energy and in a spirit of independent inquiry.

Turning to the subject of chemotherapy in tuberculosis, it should be plain that the term is used here and can only be advantageously used in the restricted sense in which it was introduced and employed by Ehrlich—not easy of concise definition—but conveying the implication of biological experimentation carefully co-ordinated with constructive chemical manipulation and even, if necessary, chemical research of a most advanced type. This use of the term is emphasized because of late the word

chemotherapy has become fashionable as applied to the empirical therapeutic use of any chemical substance from arsenic for gastric ulcer to zinc sulphate for dysentery; a use chiefly designed to conceal an utter absence of either thought or intelligent experimentation. Employing the term in the restricted sense I hope to do little more this evening than to show that it may be properly employed in connection with research in tuberculosis. Even in this I can barely carry conviction as measured by any definite success in the treatment of experimental tuberculosis.

Historical considerations on the other hand would allow to the worker in tuberculosis, if to anyone, a certain proprietary freedom in the employment of this word—and especially the real conception it represents. Ehrlich once stated that he counted as most memorable the evening on which he heard Robert Koch describe to the Berlin Physiological Society his researches culminating in the discovery of the tubercle bacillus.* Koch after this discovery at once applied himself with characteristic vigor to the discovery of a disinfectant which should act to rid the body of this destructive invader. The idea of a chemical disinfection of the body was prominent in the early writings of von Behring. As Behring and Ehrlich were at this time most closely associated with Koch (you will recall that it was Ehrlich who first showed how the tubercle bacillus could be easily stained), who can doubt that this idea was a dominant one and shared more or less equally by all three in those days. All were involved—each in his separate way in the epoch making discoveries in the realm of immunization, the theoretical development of serum therapy, and its practical employment. Ehrlich alone persisted—by virtue no doubt of his particular inclinations—in the original idea, and finally achieved a surpassing success;

* "Es war in einem kleinem Raum der Physiologischen Instituts, als Koch in schlichten und klaren Worten unter Vorlegung zahlloser Präparate und Beweisstücke die Ätiologie der Tuberkulose mit überzeugender Kraft darlegte. Jeder, der diesem Vortrage beigewoehnt hatte, war ergriffen, und ich muss sagen, dass mir jener Abend stets also mein grössts wissenschaftliches Erlebnis in Erinnerung geblieben ist." Ehrlich, "Robert Koch," Frankfurter Zeitung, June 2, 1910.

rather we should say, a series of surpassing successes each a distinct advance in one or more particulars over its predecessors. This early idea then, of a chemical disinfection of the body, let us examine it somewhat more closely as to what it was at its inception supposed to involve—and then see what that was essential had to be added to it in order to arrive at a successful result.

In Koch's writing and in those of v. Behring of somewhat later date, it was considered possible that any disinfectant might probably act in a favorable way on bacterial infection. Many substances were examined for their disinfectant action and those found active in this sense were tried on the animal in the face of the experimental disease. Koch soon emphasized as an *a priori* consideration—the probability that a substance need not actually kill the bacteria in the body—if it would only restrain the growth of the parasite, the natural body defenses might be sufficiently aided so that cure could be attained. But even with this limitation, every attempt resulted in disappointment; the stumbling block apparently being the relatively high toxicity of the chemicals tried for the complex host as against the more limited organization of the parasite.

In a paper by Boer,[1] an assistant of Behring's in this early period, is found the germ of a most important development of this early and rather crude conception. Boer found in the course of an examination of a series of substances for their disinfectant action against a number of species of bacteria, that most of them acted with a certain intensity which was of equal value against all the bacteria tried. Thus, phenol is weaker than bichloride of mercury and is weaker in the same degree no matter which micro-organism is used as a test object. Methylviolet, on the contrary, was found to be seven times more active against *B. anthracis* than against *B. diphtheriæ*. These two bacterial species being equally susceptible to phenol, mercuric chlorid and a number of other common disinfectants, it is evident that in a very limited sense methylviolet is a specific disinfectant for *B. anthracis;* to apply a modern term to an old observation.

When Ehrlich after the immunity period again turned his

attention to chemical disinfectants he was naturally interested in the study of those substances exhibiting specific activities. Working with Bechold,[2, 3, 4] he studied a series of halogen derivatives of phenol and naphthol from this point of view. Very striking instances of this "partial specificity" as they designated it were uncovered. I shall not go into detail at this time but it was shown that rather trivial chemical modifications could profoundly alter the specific features of disinfectant action. Similar series of closely related chemical compounds have since been studied from the same viewpoint by others—notably by Jacobs, Heidelberger, Amoss and Bull, at the Rockefeller Institute.

At present, work of this nature is somewhat in discredit because of the profound disappointment which has resulted from most attempts to apply substances showing exquisite action in the test tube to the more complicated situation they face when exposed in the animal body. I believe that such discouragement is premature. We are forced to admit now that which we must have foreseen, that test tube action and "in vivo" disinfection do not go hand in hand. But of all the guides available to the intimate relation of chemical agent to parasite, the study of the specific qualities of disinfectant action is the most logical and I venture to predict that in due time in some quarter, an important success will be credited to the application of this fundamental conception. In fact, if we may accept the report of Schiemann [5] such success has already been achieved in its essentials. It is stated by this observer that salvarsan, showing limited curative properties against experimental infections with the anthrax bacillus and the bacillus of swine erysipelas, has definite specific disinfectant action against these bacteria as contrasted with other species whose infections are not therapeutically influenced.

Let us leave for the moment this question of the application of *in vitro* disinfectants to the problem and turn to the first actual success achieved by Ehrlich in the treatment of an experimental infection. The benzidin dye, trypan-red, was found capable of curing trypanosomatous infection in mice. Trypan-red has very little if any action in shortening the life of the trypanosomata when the exposure is made in the test tube. It there-

fore became apparent that success in this field could be achieved by leaving aside the disinfectant qualities of substances *in vitro* and proceeding at once to the crucial test of the animal experiment with a wide variety of compounds.

If now we ask ourselves why those substances acting as disinfectants in a somewhat specific sense, fail to rid the animal body of bacteria, while other substances, hardly disinfectants in any sense, are therapeutically active in a more or less specific way, the answer is in part obvious and involves important principles which must be kept constantly in mind in all chemotherapeutic experimentation. When substances active *in vitro,* fail *in vivo,* it is frequently because they are so distributed when introduced into the animal body that they fail to come in contact with the parasite at all. The substance is quickly eliminated in some cases, is quickly and completely destroyed in other cases, has in still other instances higher affinities for the constituents of the body fluids or cells than it has for the micro-organism in question, affinities which may or may not be put in evidence by actual acute or chronic intoxication. When on the other hand apparently indifferent substances are found to be therapeutically active, it is because in the chemical melting pot of the animal body new compounds, active and with the required distribution co-efficient, are produced either from the substance itself or under its influence. These two series of qualities of substances, their capacity for specific antiparasitic action and their relation to the complexities of the animal body as evidenced by what can be found out or inferred in regard to their distribution in the body, are basic in the conception of chemotherapy.

When in any particular instance an attempt is made to elucidate these fundamental qualities, purely technical questions of decisive importance come into the foreground. Again a consideration of Ehrlich's work shows most clearly what is required. It is apparent in the first place that only those diseases can be approached in this way in which the fundamental etiological relationships are cleared up with such results that we are left with a very precise method of experimentation involving in the animal the essential features at least of the pathology of the

human disease. The trypanosomiases as studied by Ehrlich are almost ideal from this point of view. The local lesions in tissues are not prominent, the infection can be made to run a rapid and invariable course, the progress of the disease can be precisely followed from day to day by a very simple microscopic examination of the blood, and the animals of choice are small, inexpensive to purchase and maintain. If these cases are defective—and I think they are in some measure—it is in that, in the pathology of the experimental disease localization of the parasite in particular tissues does not occur as it frequently does in the spontaneous disease and there is little local tissue reaction. Questions involving the distribution of the chemical agents are consequently much more simple. It is possibly because of this fact that the experimental results have not been duplicated in a striking way in their practical therapeutic application to such conditions as sleeping sickness.

Finally in the person, in the surpassing mental attainments, of Ehrlich, another essential limitation to this form of experimentation was overcome. Co-operation so essential to achievement in every walk of modern life is more apt to materialize as between the various functional units of one man's mind than as between individuals. The united efforts of a trained chemist and a highly accomplished biologist were assured beyond possibility of even temporarily strained relations by the peculiar qualifications of this man of genius. The progress of chemotherapeutic research is contingent on a similar happy conjunction in the human mind and hands of accomplishments in these widely separated fields of research. Whether the limitation which the subject faces in consequence will in the future be surmounted by activity of individuals trained in selected parts of both fields or by the mutual and co-operative activity of specialists in the separate sciences can only be left to the chances of the future.

Having so far considered the place of chemotherapy as related to the various other possibilities of research in tuberculosis and spent some time in reviewing the origin and the more essential features of this methodical procedure, I turn to the immediate subject of the hour. I wish very briefly to consider the facts of

experimental tuberculosis in order to show what are its disadvantages and its advantages as a basis for chemotherapeutic study. Then from the work of others and from my own experience I shall draw some concrete and illustrative examples of what has been or may be accomplished.

The tubercle bacillus as cultivated in the laboratory occurs in three types showing constant qualities: the human, the bovine, and the avian. With the latter we are not now concerned. With either the human or the bovine type, most warm blooded animals, all those certainly that are commonly used in the laboratory, may be infected and the infection will run a regular course, terminating in death in many instances. The experimental disease certainly involves the essential features of the human disease. At first sight then, the requisite conditions underlying therapeutic experimentation are already at hand. I say at first sight because, in the minds of the general medical public and those laboratory workers whose experience with tuberculosis is casual. relative certainty of result speaking generally, has been exalted to an almost mythical conception of absolute certainty in each particular case, a fundamental misconception responsible for disappointing mistakes and perhaps actual harm.

Any single culture of the tubercle bacillus is a relatively constant quantity; but as in all other species of pathogenic bacteria, individual cultures differ in particulars which are of critical value when they are to be used for advanced experimentation. Attempts to modify the properties of particular cultures in any given or constant direction as a matter of controlled experiment have for the most part failed, yet over periods of years and due to conditions accidentally arising a culture may profoundly change its qualities. Thus Krauss has recently conducted certain experiments which depended upon his possession in the laboratories at Saranac Lake of cultures showing constant differences in virulence, particularly one which now produces in guinea pigs a disease which progresses for a period and then tends to heal spontaneously. With any given culture at a given time the amount inoculated and the manner in which the inoculation is made are of decisive importance to the result. With a certain culture of human type in a recent experiment of my own, $^1/_{10}$ mg.

was inoculated intraperitonially into 15 guinea pigs. The first of these died in 18 days, the fourteenth died on the thirty-sixth day and the last one lingered until the sixty-fifth day. In a subsequent experiment 14 guinea pigs were inoculated in the same way with one-half the amount of the same culture ($^1/_{20}$ mg.). Of these, the first died on the twentieth day; eight were alive on the fortieth day; four remained on the sixtieth day; and two still live on the ninetieth day. If the amount of this culture inoculated were still further reduced, we would doubtless come to a point when some animals would die and some would live for an indefinite time or perhaps recover entirely from the disease. By inoculating larger doses of this culture, no doubt it would be possible to arrange an experiment in which all of the animals would be dead by the end of four weeks.

These results conversely stated, emphasize the fact that with the same amount of culture inoculated in the same way, there is a great variation in the length of time the animals may be expected to survive. With guinea pigs I have yet to conduct such an experiment in which the last animal did not live at least twice as long as the first to die and often the difference is much greater than this. If the inoculation is made subcutaneously, this variation is increased. If it is made intravenously in guinea pigs, it is apparently not greatly, if at all, diminished. By attention to the weight and age of the animals, the variability in result as to individuals is kept at a minimum, but it is always a large factor. These facts make it plain that any experiment designed to test the therapeutic activity of a substance must be so arranged as to permit of statistical interpretation finally. The probable individual variation must be completely accounted for in each experiment. This means that a considerable number of animals must be used for each substance brought under consideration and at least as many for the requisite checks. Just how large this unit group should be has probably not been satisfactorily determined even yet.

You will have noted that the actual time involved is considerable. Thirty days is no short period and ninety days is one-third of the conventional laboratory year. Contrast this with the four

days of the typical animal experiment in Ehrlich's work with trypanosomata and it is self evident that we have here a very distinct handicap on the application of the chemotherapeutic idea to tuberculosis work.

Efforts have been repeatedly made to discount in whole or in part the variation in the length of time animals live after such inoculations by terminating the experiment on a certain date through slaughter and post mortem examination. Post mortem examination of the animals dying spontaneously reveals a variation in the exact condition of the organs which is fully as great and in my opinion considerably greater than that shown in the length of life in days. One animal dies because the liver is completely degenerated, another because the lungs are consolidated, a third is drowned in his own fluids, showing more or less local disease widely distributed, an intense oedema of the lungs and the pleural cavities completely filled with fluid exudate. These conditions, if they are to be interpreted, must also be considered statistically and this if it is to be done, must involve an even larger material than when the resistance of the animal is accounted for as a single factor. Such attempts at a short cut on any scale so far attempted are quite unsound. In any experiment —after all of a sufficient group of controls are dead and accounted for—not before then, a considerable amount of guidance may in the future be obtained from the condition of the organs of killed or dying animals.

Similarly, reliance has been placed on the presence or absence of large numbers of tubercle bacilli in the lesions or apparently healthy tissues of animals dying within the period of life of controls. If an animal dying in this period shows few lesions or none and if tubercle bacilli are found only in small numbers, it may sometimes be safe to conclude that death was not due to tuberculosis and the animal can properly be excluded from consideration. But in the presence of moderately extensive lesions it must be assumed that an animal may be as apt to die from the consequence of a largely successful effort to remove bacilli, as from the presence of the micro-organism *per se.* After the last control has died the presence or absence of bacilli may have great sig-

nificance in the experiment. Previous to that time—at least until much more extensive observations as to the variability of this factor have been made, opinion based on this point has no value.

Two things then—the absolute time involved in each experiment and the size of the experiment as conditioned by the number of animals which must be used to cover individual variations are the essential factors of difficulty that stand in the way of the application of the principles previously considered to tuberculosis as an experimental disease. Can the difficulties so imposed be directly overcome? As abstractions vary easily; as a practical matter it may easily involve a concentration of trained effort and an expenditure of money beyond that so far applied to the study of any single problem in medical science to assure an even chance of success.

It has seemed to me quite possible that the issue presented might be treated by avoiding it. I have spent a considerable amount of time in the study of various forms of local tuberculosis and of general tuberculosis in animals other than the usual guinea pigs and rabbits, in the hope of encountering a form of the disease which would be less variable or run a shorter course than I have indicated. Most hopeful for a time seemed the study of a tuberculosis of the cornea in rabbits. With Dr. Montgomery I studied rather more in detail than had previously been done this interesting local disease. On a small scale it seemed ideal for our purpose. When however the test came to be made more largely, it was found that the individual variation in reaction was as great as in other forms of experiment and that certain other factors were introduced which added to the difficulty. We were dealing here with an open lesion and this very frequently became infected with extraneous organisms. Moreover, there is a constant discharge of bacilli from the purulent conjunctival sac and unless the experiments can be segregated, the animals being cared for by already tuberculous persons, there is the constant likelihood of complicating other experiments in progress and a continuous menace to the health of one's employees. The possibilities of similar studies of other lesions or of the disease in other

animals are not at all exhausted and such efforts should prove profitable in the future.

Offsetting the disadvantages which have been pointed out as inherent, tuberculosis offers an interesting opportunity quite peculiar to itself. The multiplication of the bacillus in typical instances is accompanied by a local rearrangement of the body cells to form nodular lesions readily visible to the naked eye and of characteristic structure microscopically. These nodules, the tubercles, can be used as a guide in the study of the distribution of extraneous chemical compounds to the neighborhood at least of the bacteria. The tubercles if necessary can be picked out and examined chemically, or more practically for a beginning, colored substances can be chosen as the starting point of combined chemical and biological research. These can under favorable circumstances be immediately recognized in the tissues and questions of distribution can be rapidly answered by their use.

On the occasion of a visit to Baltimore four years ago, I was shown some very beautiful preparations by Dr. Winternitz, preparations made in a study of the origin of the cells taking part in the reaction of the earliest hours after an infection of the animal with tubercle bacilli, and since carefully described by Bowman, Winternitz, and Evans.[6] My interest was aroused in the possibility of making a wider application of the so-called vital stains and I shortly found by experiment that the fibrocaseous tubercle took up certain of these stains in a characteristic way. The results of these early experiments were recorded and summarized in the following terms:

" The first of these experiments shows again the selective action of Isaminblau for the large mononuclear phagocytic cell as pointed out by Goldman. These cells are found abundantly in the peripheral portions of fibroid tubercles.

" The second experiment is of great interest, showing as it does conclusively, that extraneous chemical substances of proper constitution may within a few days penetrate to the caseous center of a tuberculous mass and become concentrated there in greater degree than in the normal surrounding tissues. The particular substance used in this experiment, Trypan-rot, may probably be

without effect on the lesion itself, but the result should be a great stimulus to the future work in a similar direction." [7]

These experiments seemed to me to be of the utmost importance as a basis for further experimental work of a co-ordinated chemical and biological nature. The opinion had been quite generally expressed that as the tuberculous tissue was without internal blood supply it could be reached only with difficulty if at all by medicinal agents which might be made to circulate in the blood stream. Such an opinion, if of decisive weight, would make it appear to be an unreasonable waste to experiment largely with the idea of developing general medicines which might be hoped to influence the local tuberculous process. The actual result on the contrary, rendered such experimentation reasonable and as it seemed to us, eminently desirable.

DeWitt [8] in this country and v. Linden [9] abroad reported somewhat later on similar observations made independently with methylene blue. DeWitt confirmed my particular observations, reported on a number of dyes which failed to appear in the tubercle and added trypan-blue to the list of penetrating substances. I have since found, in the course of observations, so far unpublished for the most part, that a considerable number of azo-dyes related in their chemical constitution to trypan-red and trypan-blue penetrate the tubercle in this way. DeWitt has since used methylene blue as the starting point for a series of studies intended to serve the same purpose as those I am about to consider.

These observations served as the starting point of a series of researches in our laboratory which are being continued at the present time with the object of finding or building up substances which shall have this penetrating quality well developed, and which shall at the same time be possessed of physiological activity of such nature that they might be expected to act favorably on the tuberculous process. If this effort should result in any measure of success, the credit would be shared by me with Mr. Robert B. Krauss, who has been continuously associated with me for four years and who has been responsible for all of the purely chemical work carried out.

The first concrete problem outlined on the basis of the considerations above outlined was to take the dye "Trypan-rot," which had been used in the more striking of the experiments commented on, and try by chemical manipulation to form from it, or with it, a substance having physiologic activity while preserving the qualities which enabled the dye to penetrate the tubercle.

Mr. Krauss undertook to make as many modifications of trypan red as he could, following certain general lines.

1. The staining qualities of the substances were to be at least in part preserved.

2. The preference was to be given to substances containing iodine, phenolic substances, or certain other constituents.

These specifications were drawn up on very general grounds some of which may be stated as follows: iodine was selected because of the long standing belief among medical men that iodine was of some favorable influence on the progress of tuberculosis when applied locally either as an element or as iodoform; carbolic acid, guaiacol and other phenolic substances were known to be relatively active disinfectants against the tubercle bacillus and some of them also enjoyed a reputation as medicines for this disease. The staining qualities were to be preserved as far as possible as a guide to the localization in the course of the subsequent animal experimentation.

The chemical work involved was successful. In the course of a year and a half, about seventy-five compounds were secured on the plan outlined. The chemical manipulations and results were published by Krauss.[10] These preparations were used in a preliminary way in animal experiments and some interesting observations were made. In general it was indicated that none of the substances exerted any curative influence on experimental tuberculosis. Some of them seemed however to have a definite influence on the vigor and rate of the formation of blood vessels and connective tissue in and around the tuberculous process; the tests being made on the rabbit cornea.

The plan had been to prepare enough of each substance for a moderate amount of preliminary experimentation and then to

make again those which seemed useful for further work. About the time the preliminary study of these compounds was completed, the war arose and necessitated a change of plan. We had been purchasing trypan-red from abroad and further regular supply was cut off. What was on hand and the occasional lots since secured we have used in a study, so far uncompleted, of the composition of the substance with view to its manufacture.

I have already said that following our observation that the tuberculous tissue in the living animal was easily penetrated by trypan-red, many other dyes of the same general class, chemically speaking, were tested for their reaction in this particular. A considerable number were found which could become concentrated in the diseased tissue to a greater or less extent. Many of these had never been accurately described from a chemical point of view, others presented difficulty of manufacture beyond our means. After much consideration, a dye known to the trade as Niagara Blue 2B (Benzidin 2 H. Acid) was chosen as the starting point of a second attempt to construct a series of iodine and phenol compounds. It has been necessary, owing to the state of the market, for us to build up this substance from the raw products. This has been accomplished and a considerable series of compounds made by adding iodin and phenolic substances to Niagara Blue have been studied, with rather meager results which I shall not comment on now.

Covering about the same period of time as these studies on the relation of the vital stains to the diseased tissue, and in the hope of acquiring information which should in some measure guide that work, we have been studying the disinfectant action of various substances for the tubercle bacillus. We have hoped to uncover substances having the partially specific action discussed in the earlier paragraphs of this paper. Much time has been spent over methods. The procedure by which the results here considered were obtained was to determine the least concentration of the substance in glycerin-bouillon which would definitely inhibit the growth of the tubercle bacillus.

Because of the relation to the work outlined in considering the subject of the vital stains, we have so far paid particular

attention to anilin dyes. We have also considered the more common disinfectants and some substances which are used in the building up dyes. In order to get an idea of the specific qualities of the inhibiting action of the substances tested, the typhoid bacillus was at first used in comparison with the tubercle bacillus. Very recently in the course of some work being carried out for the Pneumonia Commission of the City of Philadelphia, I have gone over the whole field again, using the Pneumococcus and Staphylococcus aureus. Several hundred substances have in this way been examined with the following results:

I. The growth of the typhoid bacillus is, with few exceptions if any, less readily inhibited by anilin dyes than that of the pneumococcus, staphylococcus aureus, or the tubercle bacillus.

II. The triphenylmethane dyes, as a group, inhibit the growth of the pneumococcus and staphylococcus in dilutions which do not inhibit the tubercle bacillus. Among themselves these dyes vary greatly in the concentrations at which the four species are affected—both absolutely and relatively.

III. The azo-dyes as a group inhibit the growth of the tubercle bacillus more readily than that of either pneumococcus or staphylococcus aureus. Again there is a great relative and absolute variation in the effective concentrations. In the extreme instances in this group, the tubercle bacillus is one or two hundred times as susceptible as the pneumococcus and it seems proper to consider that the inhibitory power is in large measure specific.

IV. The other great groups of dyes give less striking results—the oxazines and thiazines to which methylene blue belongs are more active against the cocci; the eurhodines with neutral red as an example are more effective against the tubercle bacillus.

Certain other points developed by this work deserve passing mention. Many dyes, the majority perhaps stain the growing membrane of the tubercle bacillus with greater or less intensity. The inhibitory power of the dyes is not at all however a function of this capacity to stain the membrane, nor is it a function of any particular staining quality of the dyes for silk, wool, or cotton, so far as we have been able to determine up to the present; nor of the solubility of the dye in alcohol or oil as compared to

water. A small amount of work so far carried out indicate that among organic compounds other than the dyes, substances exist showing the same variable capacity to restrain the growth of the tubercle bacillus. Also a very limited number of experiments indicate that this capacity to restrain growth is not directly related according to any uniform or simple rule to the true disinfectant or lethal action of substances. Much more work will be required before these observations can be interpreted in terms of previous work with disinfectants.

Thus, approaching our problem from one point of view we have found a number of substances capable of penetrating tuberculous tissue and consequently having, as it seems to us, at least a slightly better chance of acting as anti-bacterial agents in tuberculosis than those which cannot so penetrate the diseased tissue. From another point of view, we have found many substances possessing in marked degree and in a partially specific way, the ability to greatly restrain the growth of the tubercle bacillus in the very limited conditions of the test tube.

It has for some time back been a matter of serious effort with us to bring these two sets of qualities together in the same substance. We have had a definite though limited success in this effort. The dye Niagara Blue 2B can be made to enter into chemical combination with various substances: iodin, phenol, certain fatty acids, and the like. The resulting products are apparently definite chemical compounds. As a rule they are dyes, and with properties noticably different in one or more particulars from the original substance. As a general thing, the inhibitory capacity against the cultures is distinctly increased; the capacity to enter the tuberculous tissue is on the contrary usually lost entirely or greatly diminished. In one instance so far, a condensation product of Niagara blue with formic acid—the partially specific inhibitory value is raised about twenty times, from $^{1}/_{5000}$ to $^{1}/_{100,000}$ the staining qualities for tuberculous tissues being in large measure retained.

Again, in studying the inhibitory power of various dyes, Mr. Krauss has constructed a long series of compounds, not new in principle but for the most part never before made, according

to the following scheme: Benzidin monosulphonic acid is so treated as to diazotize its free NH_2 groups. There is then added under proper conditions a quantity of the variant substance calculated to react completely with one of the NH_2 groups. When the reaction is complete there is added sufficient amidonaphthol-disulphonic acid (H) to react completely with the other NH_2 group. In this series we have also encountered a number of dyes possessing distinct inhibitory powers. Very few members of the series, so far as it has yet been extended, have any staining qualities as applied to the tissues of the living animal. When crude creosote is used as the critical component however, a mixture of closely related dyes is produced in which both qualities are present in marked degree. These products are violet blue dyes, staining the connective tissues of the living animal with moderate intensity and penetrating the tubercles very well. The inhibitory power of the mixture is stated in our terminology at $^1/_{100,000}$ or about 100 times that of the dyes trypan-red and trypan-blue which were the starting point of our work in this direction. Partial specificity is also manifested by this preparation.

The accomplishments of our laboratory up to the present in the endeavor to study tuberculosis from the chemotherapeutic point of view can then be summarized in a sentence; we have built up several substances which in the test tube are capable of restraining the growth of the tubercle bacillus in marked and measureably specific degree, and which when injected into the living tuberculous animal are capable of penetrating to the center of the masses of diseased tissue.

Attractive as may be the plan by which these substances have been reached, it would still be only the happiest accident if their further qualities were such as to render them active against the progress of tuberculosis in the animal body. Neither would the failure of two such substances mean that the idea is fruitless. For the present therefore, our efforts in the chemical laboratory are directed toward increasing the number of substances possessing these (as it seems to us) fundamentally desirable qualities. We have also turned ourselves seriously to the testing of the possibilities of these substances, and their action as therapeutic

agents. The latter phase of our work is scarcely begun and I shall pass it by with but few words.

Drawing on the literature for precedents, I may cite the following studies as the most striking of the available examples.

1. Koch and his pupils in repeated experiments during the years 1890–1897 demonstrated that by the use of tuberculin the life of guinea pigs, infected with considerable amounts of pure cultures of the tubercle bacillus, could be definitely prolonged.

These tuberculin experiments are worthy of the most serious consideration. The same result was reported by a number of different observers at the time and I find no contradictory experiments. The exact chemical nature of tuberculin has never been determined and it is to be presumed that its action comes within the field of immunity reactions rather than that of chemotherapy. I have included this substance in this brief discussion because I believe that the results with it are the best available standard on the basis of which the value of other results may be estimated.

2. Von Linden [9] in 1912 and again in 1915 presented tables showing that by the use of compounds or mixtures containing copper, or copper and methylene blue; or copper, lecithin, and cod liver oil as a salve, with or without the separate administration of iodized methylene blue, the life of tuberculous guinea pigs could be prolonged. The results as presented compare favorably with those gotten earlier with tuberculin, but in no way surpass them. Attempts to repeat some of the experiments by Corper in this country failed and Selter abroad has denied their significance.

3. Certain tables published by DeWitt [11] (1914) may be interpreted as evidence that copper and mercury compounds (or salts) of trypan-blue can act favorably on the progress of tuberculosis in guinea pigs, although the author does not put forward any positive claims for them. DeWitt has since stated publicly that she had obtained more definitely favorable results with a mercury compound of new methylene blue.

4. Koga [12] (1916) believes he has attained results of value with a compound containing copper and cyanogen. On the face of the evidence again, this substance produces a result comparable to tuberculin but not surpassing it.

5. In the case of the two substances whose development in our hands I have described above, the one, that made by condensing formic acid with Niagara Blue, has done only harm in guinea pig experiments so far. In this it corresponds to Niagara Blue itself. The diazo creosote compound seems in preliminary experiments to be capable of extending the period of life of infected guinea pigs in certain instances. The percentage of animals favorably affected in any series is less than in the tuberculin experiments of Koch's co-workers.

Those critically inclined need not search far in the original accounts given of any of these experiments to find ground for denying that they are of significance. Such criticism has been offered and will certainly be continually forthcoming. In so far as attempts have been made or are being made to introduce any of these substances into the practise of medicine, any amount of skeptical opposition is in my opinion fully warranted. In 1890 it was quite justifiable to take the first favorable results obtained in the laboratory and make careful clinical trial of tuberculin. We should however profit by this experience and proceed with great caution in the clinic until the evidence shows that new substances are distinctly better than tuberculin experimentally.

In the interest of scientific progress, on the other hand, all of these results should be treated with great charity. In view of the purely statistical nature of the inquiry, the experiments must be repeated a number of times before they are finally accepted as evidence, but it must be remembered that a single failure has no more interest than a single success. If it be granted as a probability that some measure of the truth may lie in these observations, the future of chemotherapeutic studies in tuberculosis is not discouraging. At this stage of development the possibilities compare favorably in this particular case with those which offered for the broader subject of chemotherapy when Ehrlich was first able to cure trypanosomatous mice with trypan-red. Several substances seem now to be known which are capable of giving a slight advantage to the host in its contest with the parasite. This number must be multiplied by empirical search through known chemical compounds, and each such substance

showing a suggestive lead must be subjected to carefully considered chemical manipulation to develop to the full its latent possibilities. Each year of the five that I have been actively engaged in this work has seen some added idea or suggestion of real value and we may be sure that if, in the future, faith is expressed in continuous experimentation and confidence is put in evidence by financial support adequate to the size of the task in hand, the desired result will be achieved.

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The following books contain matter of the first importance to any one interested in the development of the subject of this lecture or related questions:

a. Gesammelte Werke von Robert Koch, Leipzig, 1912.
b. Behring, Gesammelte Abhandlungen, Leipzisg, 1893.
c. Paul Ehrlich, Jena, 1914.

GROWTH CHANGES IN THE MAMMALIAN NERVOUS SYSTEM

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ON this occasion I shall limit my remarks to observations on the growth of the brain in man and in the albino rat—citing mainly results obtained by my colleagues and collaborators.

Students of growth have an immediate interest in investigations of this sort, representing as these do one chapter in a long story. At the same time the results giving the character, order and amount of these growth changes serve as norms for the pathologists and also furnish data which can be related to the behavior of the animal at successive stages of development.

The work therefore faces on several fields of larger biological interest and the significance of the observations is to be measured by the light they cast on surrounding problems.

Since the observations to be reported have been made largely on the albino rat, a word concerning the use of this animal is in order. The rat requires six days to double its weight at birth—while man requires 180 days to do the same thing; a ratio of 1–30. I have found that a rat of three years is comparable in age to a man of ninety years. Thus in both these instances the post-natal growth rate appears to be 30 times as rapid for the rat as for man.

This suggested that it would be possible to establish the equivalent ages of these two animals by the use of this ratio. The test has been made and, as we shall see, has proved successful. Thus in our studies the results obtained with the rat are compared with the data from man at the equivalent age and the high degree of accordance which appears indicates that the ratio selected—1 day to 30 days or 1 day to 1 month—is approximately correct.

It may be noted, moreover, that the method of identifying

phases in the life-span of the rat with like phases in the life-span of man, greatly increases the usefulness of the rat for laboratory purposes and gives us a new and effective method of approaching human problems by way of the animal.

In formulating what follows, several assumptions have been made. First, that we understand by the term growth, increase in volume—implying an increase in weight also. Second, that there are other changes occurring between birth and maturity that may be described as differentiations—structural or chemical. Moreover I have assumed a general knowledge of the nervous system and of the brain with its white and gray matter and of the terms neuron for the entire cell, and axon for the axis—cylinder or principal outgrowth from the cell body.

The brain like other organs is made up of cells. Consequently by a proper procedure we could examine these cells one by one and count them. The absolute number is not of so great interest to us at the moment however, as the determination whether the number found in the brain of any mammal is sufficiently constant—to be characteristic for the species. Thus at the threshold of our investigation stands a fundamental question.

At maturity the weight of both the human brain and that of the rat shows a wide range of variation and it becomes at once important to learn whether among the individuals forming a species there is a like number of constituent cells, or neurons, composing the brain, or whether the wide range in weight is associated with a wide variation in the *number* of neurons also. However, in a case like this it is hardly proper to expect a fixed number of cells—in the physical sense—but we should rather expect a "characteristic mean number" (*i.e.*, characteristic for the species) associated with an equally characteristic variability—for we must recognize that variability is always and normally present.

There are two sets of determinations—both on the rat—which bear on this question. Greenman ('17) has determined the number of myelinated fibers in the peroneal nerve of the albino rat. The results are given in Table 1.

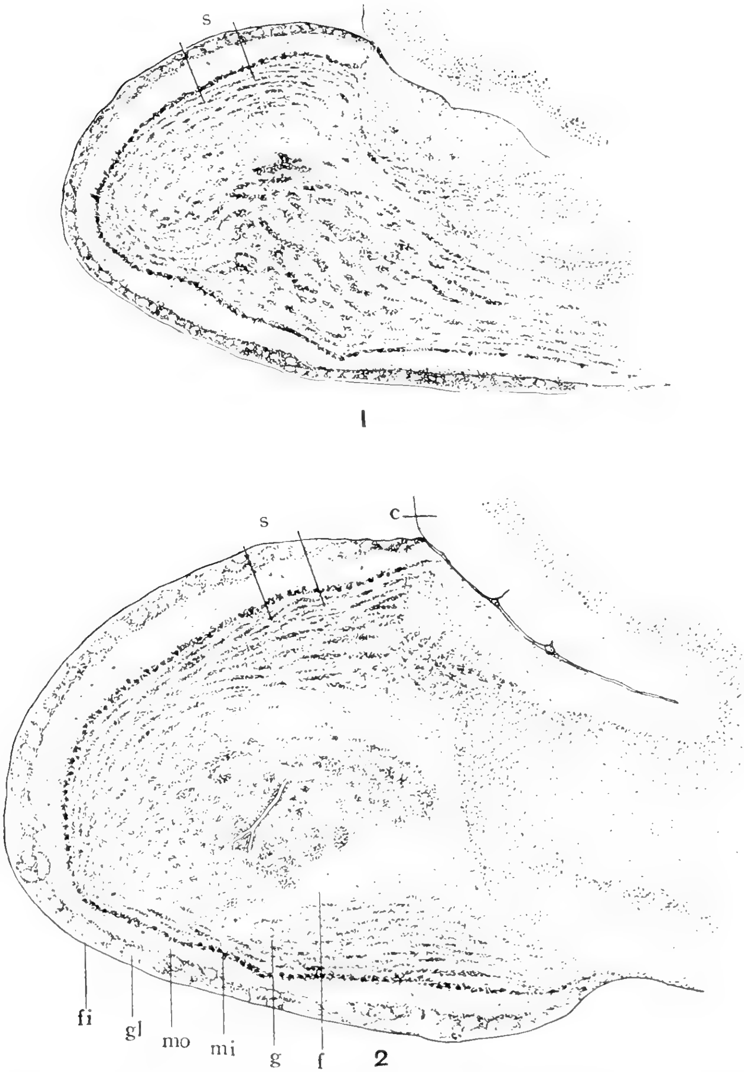

FIG 1.—Section 1. Median longitudinal section through olfactory bulb of rat underfed thirty-one days. Final brain weight, 1.5470 grammes; bulb weight, 0.0203 gramme. Defective diet. Magnified 24 diameters. Section 2. Median longitudinal section though olfactory bulb of control rat. Brain weight, 1.6984 grammes; bulb weight, 0.0315 gramme. Magnified 24 diameters. (Holt, 1917). s, areas in Figs. 1 and 2 enlarged in Figs. 3 and 4 (Holt, 1917); fi, outer fibre layer; gl, glomeruli; mo, molecular layer; mi, mitral layer; g, granular layer; f, inner fibre layer; c, cerebrum.

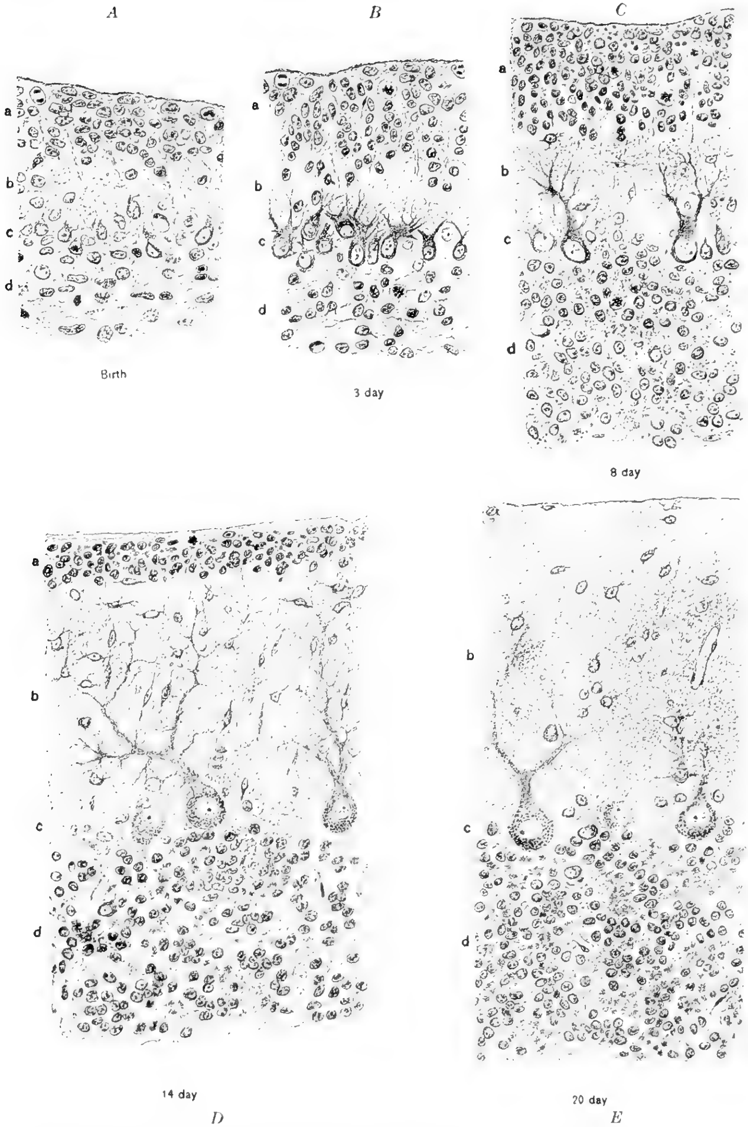

FIG. 2.—Cortical layers of cerebella of animals of different ages from median sagittal sections. All drawings made from boundary of sulcus primarius with aid of camera lucida. × 400. *A*. From newborn animal, sagittal section of cerebellum. Mitoses in outer stratum. Purkinje cells distinct, but irregularly arranged. *B*. Three days. Outer granule layer has widened. Mitoses in its outer subdivision. Purkinje cells with cytoplasm aggregated at ectal pole from which branch out several processes. *C*. Eight days. Outer granule layer at its maximum thickness. Cells compact, with mitoses. Purkinje cells are elongating to form single stout dendrite. Molecular layer widening. In it are seen both vertical and horizontal fusiform cells, also multipolar cells which are probably basket cells. Granule layer increased, and shows Golgi cells. Mitosis in granule layer. *D*. Fourteen days. Outer granule layer diminishing. Molecular layer increased. Many vertical fusiform cells migrating toward granule layers. The multipolar cells represent small stellate and basket cells. Purkinje cells show Nissl granules. Granule cells becoming grouped. Golgi cell with light nucleus, single nucleolus and thick mantle of cytoplasm. *E*. Twenty days. All outer granule cells have migrated and molecular layer has its mature appearance. Purkinje cells are spaced farther apart. Two Golgi cells are shown in the granule layer.

TABLE 1.—NUMBER OF MYELINATED FIBERS IN PERONEAL NERVE—INBRED ALBINO RATS—GREENMAN, 1917.

Group 1. 15 rats, 151 days old	Average number of myelinated fibers	Probable error of the mean
Ave. no. in 15 right peroneals	2038	± 17
Ave. no. in 15 left peroneals	2032	± 13
Group 2. 12 rats, 153 days old		
Ave. no. in 12 right peroneals	2071	± 18
Ave. no. in 12 left peroneals	2069	± 17

It is to be noted that the number of myelinated fibers tends to increase with age, and that the two series give comparable values —when the probable error of the mean is considered.

These results indicate also a correspondingly constant number of cells of origin—both efferent and afferent—from which the fibers have grown out.

The second of these studies is by Miss Holt ('17) on the olfactory bulb of the albino rat. In this instance a count was made of both the mitral cells and the small cells in the bulb, and a remarkable constancy in number was found. The layers of cells are shown in two sections in Figs. 1 and 2.

In this case the difference in brain weights is 10 percent while the difference in bulb weights is 50 percent. The numbers of cells found are given in Table 2.

TABLE 2.—CELLS IN ONE OLFACTORY BULB: ALBINO RAT. OBSERVATIONS OF DR. C. M. HOLT, ('17).

	Mean	Extremes
No. of small cells; average of 3 cases	1,000,000	± 2 per cent.
No. of mitral cells; average of 13 cases	76,749	± 9 per cent.
No. of mitral cells ... Range	(70,625–83,974)	
In five litter-pairs (10 cases) average difference of test from control	–2 per cent.	

Although the bulbs differ widely in absolute weight, it is found that the larger cell numbers do not go with the heavier bulbs. We conclude therefore that the rat has a characteristic mean number of neurons forming its nervous system. The same statement probably holds true for man. From these two studies

it is inferred that for a given species the number of cell divisions in the nervous system is characteristic and is also highly constant.

The foregoing statements about the number of neurons apply to the brain at maturity—but the brain at birth has a somewhat different constitution.

At birth cell division is still in progress, and this is true for both man and the rat; moreover, for some time after birth cell division continues—especially in the cerebellum. Allen ('12) finds in the *cerebrum* of the rat some cell division up to the twenty-fifth day of post-natal life, after which time it diminishes rapidly and soon becomes insignificant. In the cerebellum, however, post-natal cell division is more abundant than in the cerebrum and is responsible for considerable change. The enumerations are given in Table 3.

TABLE 3.—BRAIN OF ALBINO RAT. MITOSES IN ONE CUBIC MILLIMETER OF NERVE TISSUE (ALLEN '12).

	No. of mitoses Brain	
Age days	Cerebellum	Cerebrum
1	1597	430
4	2111	447
6	....	193
7	4848	...
12	839	37
20	127	23
25	00	27

What happens in the cerebellum of the rat while this cell multiplication is in progress is shown in Fig. 2 representing the work of Addison ('11).

As these studies by Addison show, during the first 20 days after birth, and especially during the first 10 days, the elements of the external granule layer are rapidly dividing and migrating to a position below the Purkinje cells. The Purkinje cells are also maturing and at 14 days some of them have attained nearly full size. Increasing control of locomotion accompanies this development of the cerebellum and at about 14 days the rat has fair locomotor control, and can return to the mother over a complicated path (Watson, '03, p. 118).

The rat brain at 5 days of age is in the same stage as the human brain at birth—therefore the conditions in the cerebellum at 20 days of rat life correspond to those at 15 months of human life, and in both man and the rat motor control runs parallel with cerebellar development and is attained at the equivalent ages (Lui, '94).

With the cessation of cell division and the establishment of final numerical relations, the growth of the brain becomes a matter of enlargement of the neurons.

When it is necessary to follow the growth of the human brain in weight from birth to maturity, it is important to remember that during the first year and a half, the growth is brought about both by cell division and by cell enlargement—while after this age the brain grows by cell enlargement alone.

Figure 3 represents the growth of the brain in weight during the first 75 years of human life. Those familiar with this topic will note that the curve or graph does not show the usual fall in weight after 15 years (this latter being indicated by the dots) but maintains its level.

This course of the graph is the result of applying a correction for the fatal illness—since the fatal illness causes a loss in brain weight. For this correction I have used as a basis the determinations by Gladstone ('05) on the effect of chronic disease on brain weight. Gladstone's data are given in Table 4.

TABLE 4.—LOSS IN HUMAN BRAIN WEIGHT AFTER CHRONIC AS COMPARED WITH ACUTE DISEASE (GLADSTONE, '05).

A = acute. C = chronic.

		MALES			FEMALES		
Age, years	Number	Weight, gms.	Loss, per cent.	Loss, per cent.	Weight, gms.	Number	Age years.
33.7	15 A	1429			1303	5 A	29.2
	42 C	1346	5.8	4.6	1243	48 C	
56.5	22 A	1318			1246	3 A	66.0
	55 C	1302	1.2	3.8	1198	47 C	

A corresponding effect of disease is shown by "rat pneumonia," a chronic ailment, on the brain of the rat. The graph

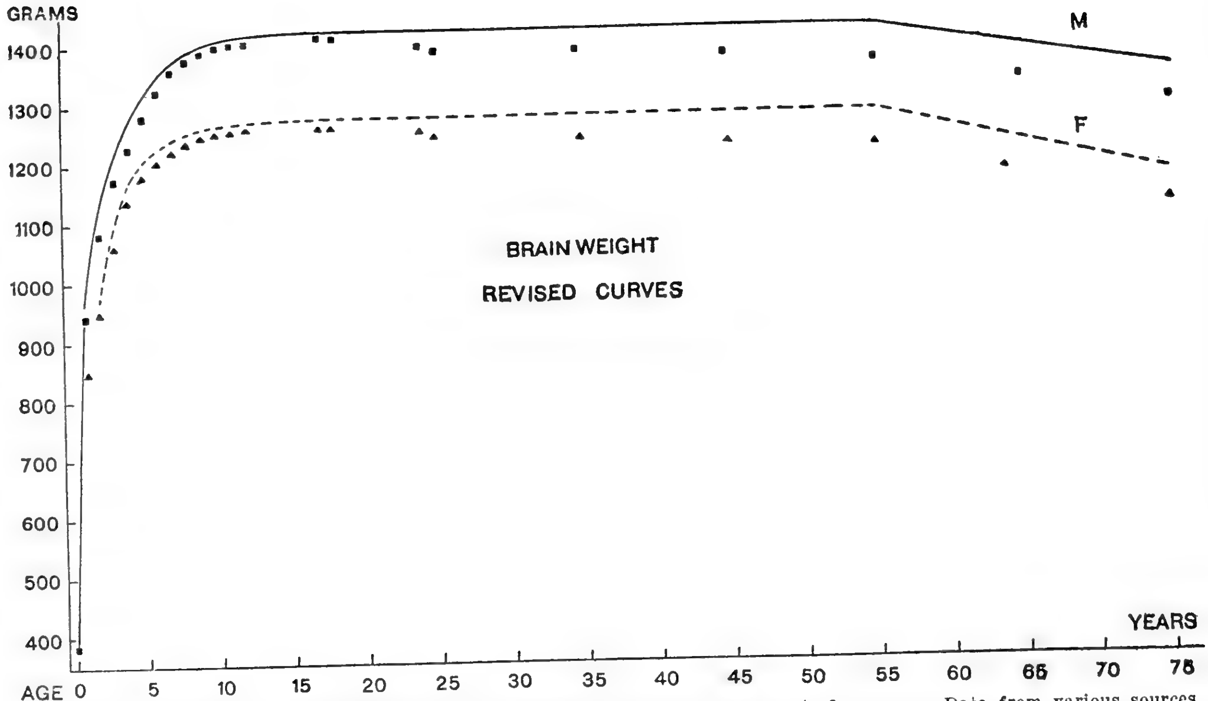

FIG. 3.—Giving the brain weight in man—on age—from birth to seventy-five years. Data from various sources. The ■ marks give observed weights for the male brain and the ▲ marks the same for the female brain. The continuous line graph gives the computed weights for the males after correction for the loss of weight due to fatal illness, and the broken line graph the corresponding weights for the female brain.

showing the increase in the weight of the rat brain on age, appears in Fig. 4. With this is compared the graph for the growth of the human brain reduced to accord with the assumptions that the human brain was growing as fast as the rat brain and that the final weights were the same for both (time relations 1–30: weight relations 1–726).

The human brain record for birth is entered at five days of rat age because the rat brain at birth is less mature than that of man.

The value of this comparison lies in showing that at equivalent ages the two brains are in nearly similar phases of growth. That the two graphs do not coincide more closely is due to several causes, one of which is the smaller relative size of the cerebral hemispheres in the rat. The correspondence is such, however, that we may look for similar changes in both brains at equivalent ages and the rat brain may therefore be used for more detailed studies, with confidence that several classes of results obtained by the aid of it can be applied to man.

If the rat brain be divided into four portions—olfactory bulbs, cerebral hemispheres, cerebellum and stem, as shown in Fig. 5, then the growth of these parts in the rat is illustrated by the graphs in Fig. 6. Touching these graphs several comments are in place:

(1) At about 20 days the cerebrum has attained half of its final weight. I shall wish to recall this fact when the growth of the cerebral cortex is considered.

(2) The growth of the stem is the most persistent.

(3) The greatest relative growth is shown by the cerebellum.

(4) The olfactory bulbs exhibit a decline in weight after the period of most rapid growth.

Problems arise in connection with all of these parts but for the moment we must neglect them.

In this connection the growth of the cerebral cortex is of interest. It is my privilege to report some unpublished observations on the growth of the cerebral cortex, by Dr. Sugita, now working at the Wistar Institute. The cerebral cortex of the rat has been sampled by making a series of thirteen measurements on it as it appears in frontal, horizontal and sagittal

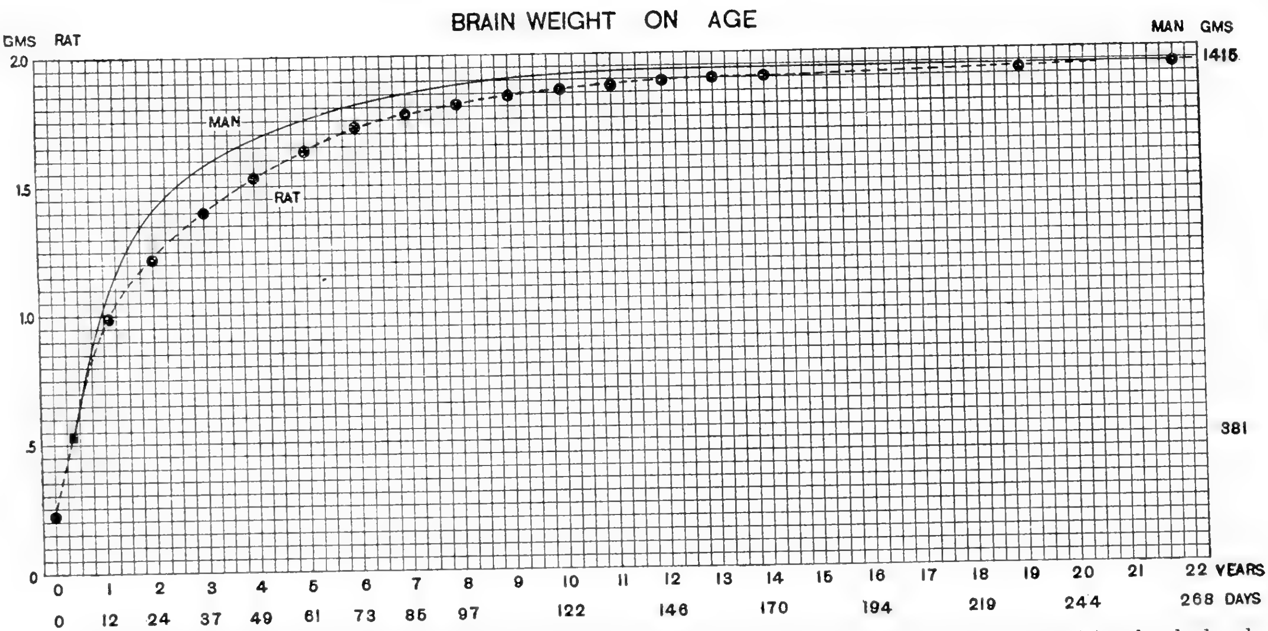

FIG. 4.—Giving in the broken line graph the brain weight of the rat in grammes, on age, for the first two hundred and ninety-two days. For comparison the brain weights for man (male), reduced by dividing the chart values (see Fig. 3) by 726, are entered at the equivalent ages—in the form of a continuous graph. The weight of the human brain at birth is entered at 5 days.

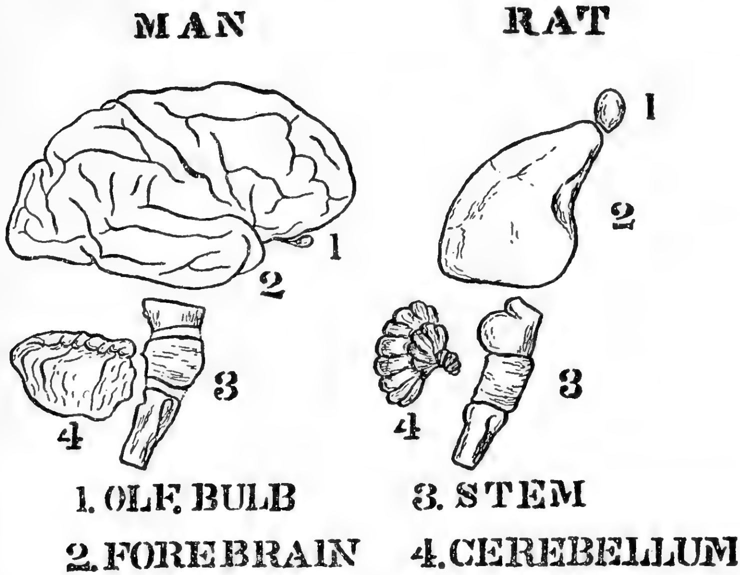

FIG. 5—Showing the human brain divided into 1 and 2, the olfactory bulbs and forebrain combined, 3, the cerebellum, and 4, the stem. In the case of the rat brain all four parts are shown separately.

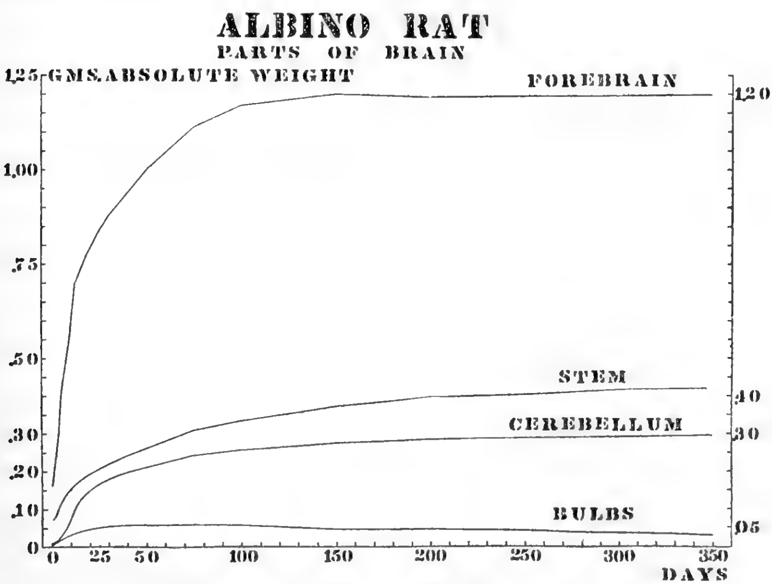

FIG. 6.—Showing the increase, on age, in weight of the forebrain, cerebellum, stem and olfactory bulbs of the albino rat.

sections. The condensed result of these thirteen measurements is given in the graph which appears in Fig. 7.

The course of this graph is most interesting. At 20 days (Br. Wt., 1.15 gms.) the cortex has attained nearly its full thickness, but at that age the cerebrum, as we have seen, weighs only about one-half as much as it will at maturity. However, when the weight doubles, the surface to be covered increases by about sixty percent, and there thus appears the phenomenon of the cortex maintaining a nearly fixed thickness while undergoing a sixty percent increase in area—a process which still requires detailed analysis. Using this determination on the rat (at 20 days) as a basis, we should expect the human cortex to reach a like phase early in the second year of life (15 months) but no careful study of this point has yet been made.

The foregoing observations on the growth of the brain during the first year, or the first third of the span of life of the rat, have been given in terms of weight, size and number, but it is possible to measure the same changes in terms of composition also.

In its most general form the composition of the brain may be expressed in terms of water and solids, a relation which changes with age. When the water and solids forming the brain of the rat are determined and the results expressed as the percentage of water in the fresh brain, there appears the graph shown in Fig. 8.

As is seen, the percentage of water falls off rapidly during the first 40 days and afterward at a much slower rate. The entire fall amounts to about 11 percent and is closely linked with age. From the literature (Weisbach, 1868–Koch and Mann, 1909) I have been able to collect four determinations of this same character in man (Donaldson, 1910) and when these are entered —by the large dots—at the equivalent ages—it is evident that they fit closely with the values obtained for the rat. This agreement once more gives support to the view that at equivalent ages the brains in the two species are in like phases.

Note, please, that the loss of water is practically complete in the phase which corresponds to 3.5 years of human age—that is at an age when the mental powers are only beginning to unfold.

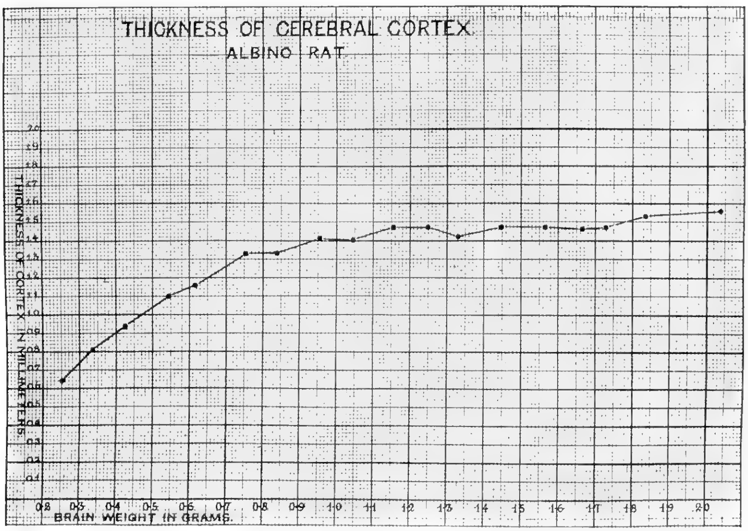

FIG. 7.—The thickness of the cerebral cortex of the albino rat given in millimetres on brain weight in grammes. For each brain weight thirteen measurements were made and more than 100 brains were so studied. In the chart the mean values are given grouped at intervals of about one-tenth of a gramme. The mean values represent the thickness as determined from measurements on the slide and without correction for the effects of the reagents used. All the brains were, however, treated in exactly the same manner (MS N. Sugita).

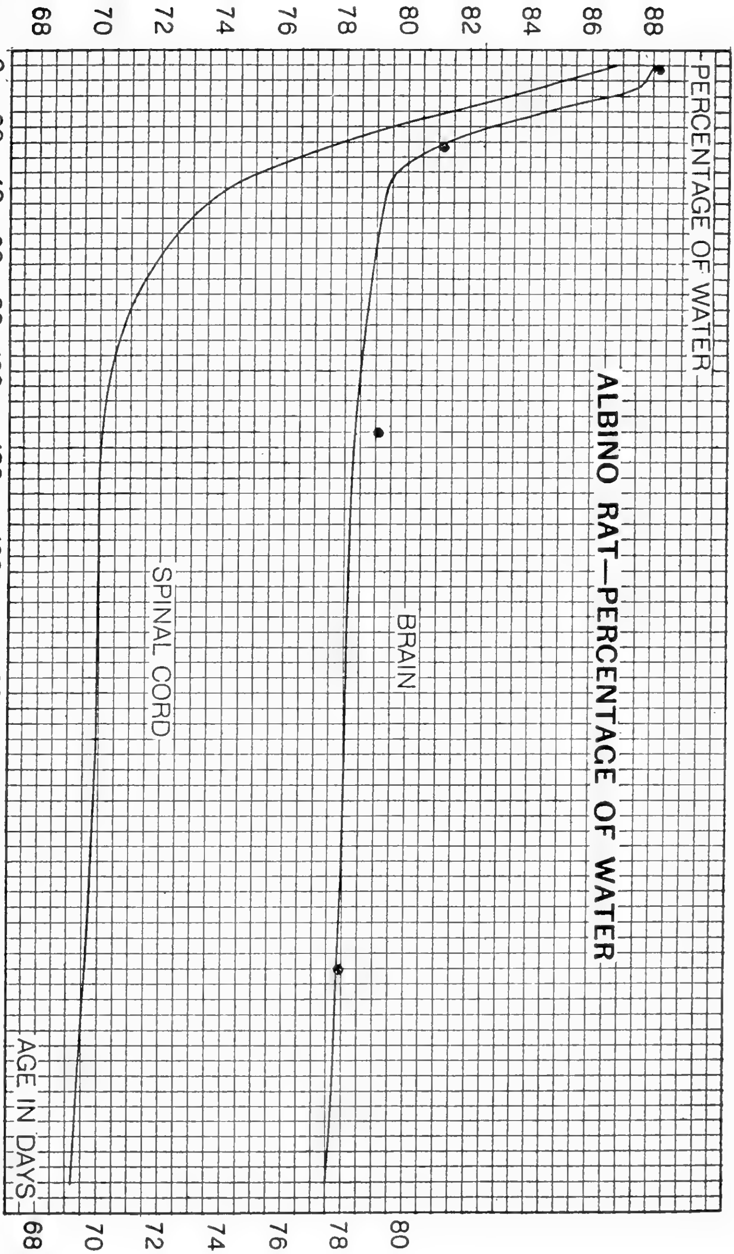

Fig. 8.—Giving for the rat, on age, the percentage of water in the brain (upper graph) and in the spinal cord (lower graph). The heavy dots (●) on the graph for the brain show the percentage of water found in the human brain at equivalent ages (Donaldson, 1910 and 1916).

Recently it has been possible to interpret this loss of water by showing how it takes place. It has generally been assumed that the loss of water in the nervous system was due to the formation of myelin—the substance which constitutes a thick sheath on many nerve fibers—because the loss occurred during sheath formation. At the same time it has not been known how the neuron—represented by its cell body and *unsheathed* axon alone—was behaving in respect to its content of water during this process.

Recently, however, by using the data gathered by the Kochs ('13) it has been possible to show for the rat brain that the progressive accumulation of myelin, which has a water content of about 48 percent, satisfactorily accounts for the observed loss of water in the brain as a whole—the water content of the neurons, in the strict sense, being modified but little, if at all (Donaldson, '16 and '16a). There is every reason to consider this conclusion as applicable to man.

Passing from the relations of water and the solids to the analysis of the solids themselves, we have the valuable study by W. Koch and M. L. Koch ('13) on "The chemical differentiation of the brain of the albino rat during growth." One of the determinations which they made is given in Fig. 9.

The protein recorded on this chart forms about 50 percent of the brain solids and the lipoids about 40 percent. The chart shows also, with the beginning of myelination, an outburst of phosphatid formation, although the cerebrosids and sulphatids are the groups most characteristic for the myelin sheath. From the varying rates at which these several groups are formed, it is seen that the composition of the myelin alters in respect of these groups with advancing age.

Leaving the growth changes as shown by the entire brain and its larger divisions, we turn now to the changes observed in the constituent neurons.

In general the growth of the cell body is characterized by an overgrowth of the cytoplasm in relation to the nucleus. Where dendrites are formed, these become more numerous with advancing age—up to a certain point. In the spinal cord, in the cerebral cortex and in the cerebellum some cell bodies in the rat reach approximately a maximum size at 20 days or earlier, while

the maximum is attained somewhat later by the cells of the spinal ganglia. The axon—the axis of the nerve fiber—is at first small in diameter and of course short when the animal is of small size. Its length increases with the growth of the animal, while in some systems of neurons at least, its diameter also increases in a remarkable way. Thus the fibers forming the ventral root of the

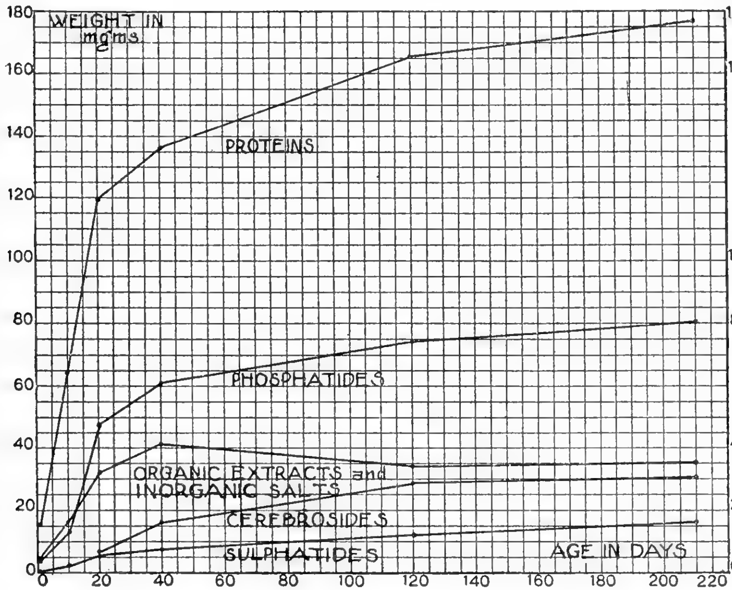

FIG. 9.—Showing the absolute weight in milligrammes of the constituents of a single brain of the albino rat at different ages (W. Koch and M. L. Koch, 1913).

second spinal nerve grow in area as indicated by Fig. 10, taken from Dunn's ('12) study.

As has been stated, some cell bodies in the spinal cord—from which these fibers come—have reached approximately their full size at about 20 days of age. The area of the cross section of the corresponding axons would at that age be somewhat greater than at 14 days (Fig. 10) say $30\mu^2$, while at maturity (270 days) the area has risen to $120\mu^2$ or four fold—the cell body remaining the same size. There is some indication that a similar process of

growth occurs in the spinal cord—but no systematic study of this phenomenon has been made. In the case of the spinal nerve root fibers the myelin sheath soon attains a volume equal to, or somewhat greater than, the enclosed axis, while under conditions not yet determined the sheath, in some of the peripheral nerves, may become relatively even thicker (Greenman, '17).

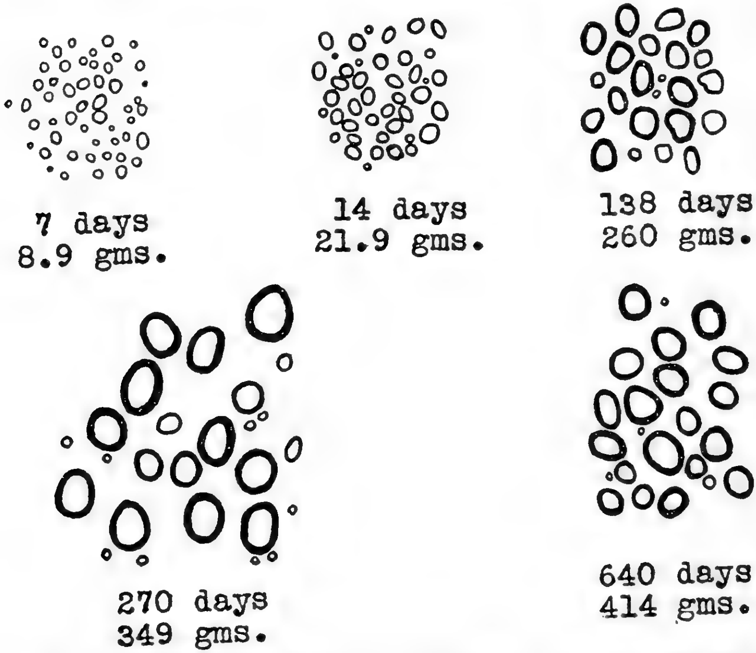

FIG. 10.—Giving at five ages the cross-sections of the largest fibres from the ventral root of the second cervical nerve of the albino rat. Osmic acid preparations. Uniform enlargement (Dunn, 1912).

From a series of unpublished studies designed to determine the age at which some of the cell bodies in the various divisions of the nervous system attain their full size, it is found that between 20 and 25 days (in the rat), cell bodies of approximately maximum size are present in the various localities. They represent, of course, but a fraction of the final number of large cells (Kaiser, 1891). Where the cell bodies are of full size, they have also established their functional connections. Thus the neurons

of a locality develop functionally, not in platoons, but in skirmish order—some much in advance of the others. As a result, a sketch plan or working model of the central system is early developed. Taking 20 days as the age in the rat when this organization is accomplished, the equivalent age for man would be 15 months. This gives a neurological basis for the fact that the developing child shows at this age, or perhaps even earlier, most of the fundamental reactions of the nervous system, but they are, as we know, uncertain, feeble, and subject to rapid fatigue. With continued growth more of the fundamental neurons mature, the significance of the correlating neurons increases, and as a result there appear, not so much new reactions, as an elaboration of those already exhibited but now performed with increased precision, strength and endurance. This condition is perfected as maturity is attained.

One inference from this is that the fundamental reactions should be exhibited *early* by individuals with a complete and normal nervous system.

Closing here the presentation of special studies, let me attempt to bring the data together in their chronological order, and assuming that all the results apply to man, to form a general picture of the course of events as they occur.

At birth the brain is composed of neurons, most of which have ceased to divide, but there are still some dividing cells, most numerous in the cerebellum. This phase is completed during the first part of the second year, and with the completion of the development of the cerebellum, comes the ability to walk. At this time the number of neurons forming the system, a number which is characteristic for the species, becomes complete and the subsequent growth of the brain depends mainly on the enlargement of the formed elements.

In the process of enlargement some cells in the several localities are in advance of others, so that the neurons develop in skirmish order, with the early formation of a working model of the nervous system and functional responses are first present in an imperfect form, to be later perfected as growth proceeds. During enlargement, the neuron changes in form. The cytoplasm

grows more rapidly than the nucleus and, where they are characteristic, dendrites are formed—accompanied by the outgrowth of the axon.

While in most localities some cell bodies reach full size early, the axon may go on growing after the cell bodies have stopped, and in the efferent spinal nerves at least, may exhibit at maturity a sectional area some four times that which it had when the cell body was already of full size.

The axon, starting naked, soon acquires a sheath which attains the same or a greater volume than the axon itself. While this process of myelination is going on, the water content of the neuron, represented by the cell body and the axon, suffers but little, if any, diminution, yet the brain as a whole drops from 88 percent to 79 percent of water during the first three and a half years of life—owing to the accumulation in the brain of the sheathing myelin, with a water content of 48 percent. The final percentage of water in the brain is about 77.5 percent. The formation of the myelin, which is probably a product of the activity of the axis cylinder, is accompanied by characteristic chemical differentiations.

The increase in the weight of the brain as a whole results from these growth changes in the constituent neurons, and its growth, as we have seen, is precocious as compared with that of the entire body. The brain weight at death is however modified by the effect of the fatal disease—so that in attempting to determine the normal brain weight, a correction must be made for this influence.

In turn the growth of the entire brain may be built up from the growth of its divisions: cerebrum, cerebellum and stem—each of which increases in weight in a characteristic way. In the normal brain the proportional weights of the parts are highly constant, and deviations from these proportional values are excellent indications of a serious departure from normal growth.

As regards the cerebral cortex, the interesting feature is the fact that the cortex has attained its thickness at 15 months of age, so that it must later undergo a large increase in area without any significant change in thickness. All this shows that the

important events in the postnatal growth of the nervous system occur early in life, and this in turn emphasizes the paramount importance of favorable conditions during the first three years of childhood.

As students of the human nervous system, our object is, I take it, not only to interpret deviations from the normal in growth, but also to control and possibly even to improve the growth process. To achieve this control or make this improvement it is necessary in the first place to find out what is taking place in the normal animal under the usual conditions, and it has been my endeavor therefore to present to you this evening some of the observations that have been made in this field.

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THE SUPPLEMENTARY DIETARY RELATIONSHIPS AMONG OUR NATURAL FOODSTUFFS

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IN order that I may illustrate the supplementary dietary relationships among certain of our naturally occurring foodstuffs, it is essential first that I offer convincing evidence that we are now in a position to devise experimental procedure which is adequate to reveal such relationships. Secondly, it must be shown that we possess sufficiently complete knowledge of the essential factors which operate in making a diet adequate for a growing animal to enable us to interpret correctly the results of feeding experiments. This can be done perhaps best by briefly reviewing the work of the past decade which has been most significant in advancing our knowledge of the chemical complexes which are indispensable to the growing mammal.

Previous to 1909 a number of attempts had been made to nourish animals on diets composed of purified proteins, carbohydrates, fats and inorganic salts usually with a small admixture of cellulose. Such attempts were attended with failure. In every instance the cause of the failure was entirely unknown. The speculations regarding the cause dwelt principally upon the dependence of the mammal upon a supply of some organically combined forms of phosphorus, as phosphoprotein, lecithin, nucleic acid; its requirement of iron in some special organic form, and with the need of purine bases preformed in the diet.[1] The first experimental work which proved fertile in advancing our knowledge regarding the factors which are essential in an adequate diet was that of Stepp.[2] He attempted to estimate the importance of lipoids in nutrition, and found that bread prepared with milk was after extraction with ether and alcohol no longer capable of maintaining life in mice. The total material

extracted by the solvents, when put back into the diet, made the food efficient once more. Stepp was unable to obtain this result by the addition of any known lipoid. The latter result is significant in the light of later developments.

In 1911 Osborne and Mendel [3] reported remarkable results in experiments involving growth in young rats which were fed a mixture of purified protein, carbohydrate, fat and 28 per cent of what they termed "protein free milk," a preparation made by removing the casein of skim milk with acid and the albumen so far as possible by coagulation with heat. The "protein free" whey was evaporated to dryness. "Protein free milk" was found to induce normal growth when supplemented with purified food ingredients, and its discoverers were inclined to attribute its virtues to a nice adjustment of the inorganic constituents of the diet, although they appreciated the possible importance of unknown constituents. Two years previously Hart, Steenbock and I [4] had reported most marked improvement in the capacity of cows fed on a ration restricted to the products of the wheat plant, to produce viable young, by suitable supplementing of the food mixture with certain inorganic salts, and this seemed at the time to be an adequate explanation of the good effects of feeding "protein free milk."

There were observations at this time available in the literature of pathology which were of great value in suggesting profitable lines of procedure to those who were engaged in studying the problems of normal nutrition during growth. In 1897 Eijkmann [5] had reported his observations on the production of experimental polyneuritis in fowls, analogous to human beriberi, by restricting them to a diet of polished rice. He further showed that undecorticated rice could induce a cure of fowls in this condition.

Fraser and Stanton [6] in 1907 had employed alcoholic extracts of rice polishings for the relief of experimental polyneuritis. In 1911 Funk [7] took up the study of the problem and showed that pressed yeast, hydrolysed with 20 per cent sulphuric acid for 24 hours, still retained its property of curing polyneuritis when administered to birds. This observation, disposed of the view.

which had been much discussed, that some organic phosphorus compound was the curative agent. All such compounds are reduced to their simplest cleavage products by such vigorous treatment. Funk and simultaneously Suzuki [8] and his co-workers in Japan showed that the substance which relieves polyneuritis is precipitated by phosphotungstic acid and is therefore an organic base.

It would be obvious to anyone who made careful inspection of the literature up to the end of 1911 that further efforts directed toward the nutrition of animals with purified food substances must be attended with failure. As early as 1906 Hopkins [9] had reported his observation that the addition of small amounts of milk to food mixtures consisting otherwise of purified protein, fats, carbohydrate and salts, exerted a favorable influence on nutrition which was out of all proportion to the calorific value of the added milk, and he expressed the belief that there existed certain "accessory food substances" indispensable from the diet. These observations convinced me of the importance of further examination of the lipoid moiety of the diet. In 1912 Miss Davis and I observed the remarkable stimulating action on growth of purified butter fat and of the ether extract of egg yolk, when these were added to a mixture of supposedly purified food substances. Lard or olive oil possessed no such properties.[10]

The situation was still not clear. In June, 1912, Osborne and Mendel [11] described experiments in which normal growth curves were secured with rats over a period of 60 days, on a ration consisting of pure protein, starch and "protein free milk," all the constituents being free from substances soluble in ether, and practically free from lipoids of any character.

One phase of the difficulty was cleared up by our observation that when lipoids of suitable character were present in the food mixture, we secured growth when 20 per cent of the diet consisted of nearly nitrogen free lactose (Merck or Kahlbaum), but were unable to do so when the latter was replaced by starch.[12] Obviously an impurity in the lactose furnished a second unknown dietary factor without which growth could not proceed. The inclusion of lactose with the omission of the fats of milk or egg

yolk was, however, attended with failure to secure sustained growth. This was difficult to understand in the light of Osborne and Mendel's temporary success, in inducing growth by the employment of ether extracted "protein free milk." The explanation is now available. Simmonds, Steenbock and I [13] have found that the fat soluble dietary essential is appreciably soluble in water, and that repeated washing of butter fat with distilled water removes from it the substance which is responsible for its peculiar effect in the diet. The water solution of the non-lipoid constituents of milk therefore contains this substance and indeed in considerable amount. Recent experiments lead us to believe that approximately half of the fat soluble dietary essential is present in the fat and half in the non-fat portion of the milk. It is therefore approximately thirty times as soluble in butter fat as in water. "Protein free milk" contains therefore both the water and alcohol-soluble dietary essential, which is found in many natural foodstuffs and which is the active agent in the cure of polyneuritis, and a small amount of the fat-soluble essential as well.

Observations such as I have described led Miss Davis and me in 1915 [12] to propose the working hypothesis that an adequate diet during growth must furnish in addition to the well recognized constituents of the diet: protein, carbohydrate, fats, and inorganic constituents of adequate character and amounts, two as yet unidentified dietary factors. One is soluble in water and alcohol and apparently never associated with the lipoids, when these are isolated from natural foodstuffs,—the other soluble in fats. The latter is extracted from milk, egg yolk, kidney and probably other animal tissues by ether, but is not removed by this solvent from either the seeds or the leaves of plants.[14] The former is universally present in foodstuffs of vegetable and animal origin, but is absent or nearly so from crystalline sugar, starch, and fats, and is present in but small amount in polished rice and probably in those foods derived from the endosperm of seeds by milling processes. Where the reaction of the medium is alkaline, strong heating of foods probably leads to its destruction.

Stepp failed to correctly interpret his observations because

hot alcohol takes out both of these dietary factors, and when he put back the extract with the extracted residue, both were restored.

Osborne and Mendel could not correctly interpret the cause of their success with "protein free milk" combined with purified foodstuffs, because they were adding both these unknown substances, although the amount of the fat-soluble was one inadequate to serve throughout the life of the animal.

I am frequently confronted with the questions: What of the several "vitamines" which have been so eagerly seized upon of late to explain such pathologic manifestations as beri-beri, scurvy, pellagra and rickets? Do not these several well recognized syndromes, the etiology of each of which is generally believed to be more or less directly referable to inadequate diet, connote the existence of as many "vitamines" each specific in the protection it affords against a type of abnormal metabolism? I am almost convinced that there are but two individual substances, the solubilities and distribution of which I have just described, which constitute the only essential dietary ingredients with whose chemical natures we are not fairly familiar. The possibility remains, of course, that one or both of what I have termed the fat-soluble A and water-soluble B each represent more than a single substance. I shall speak further on this point later.

Employing this working hypothesis we have examined the nature of the dietary deficiences of several of the more important natural foodstuffs. The procedure in each case was as follows: The individual foodstuff, maize [15] for example, was fed (a) as the sole source of nutriment. This leads to complete failure of young animals to grow, and death usually supervenes within two months. To other groups we fed (b) maize plus butter fat to furnish the unidentified dietary factor A; (c) maize plus a liberal amount of a complete protein, casein; (d) maize plus a salt mixture so constituted as to supplement the inorganic content of maize and give the animal an inorganic supply similar to that which is supplied by milk, a food on which good nutrition with growth is attained. As will appear later, maize contains a liberal amount of the water-soluble B. In all these experiments

Composition of Salt Used With Rats 223, 223 B, 380

NaCl	0.668
K_2HPO_4	0.337
$CaH_4(PO_4)_2H_2O$	0.284
$MgSO_4$ (anhydrous)	0.018
Mg citrate	0.068
Na citrate (anhyd.)	0.037
Fe lactate	0.119
K citrate	0.799
Ca lactate	2.533

Composition of Salt Mixture 318

NaCl	1.40
K_2HPO_4	2.531
K citrate H_2O	0.710
$CaSO_4$	0.578
Ca lactate	7.058

Composition of Salt 500

NaCl	0.5148
$CaCl_2$	0.2569
K_2HPO_4	0.3113
K citrate	0.5562
Ca lactate	2.878

Composition of Salt Mixture 185

NaCl	0.173
$MgSO_4$ (anhyd.)	0.266
NaH_2PO_4–H_2O	0.347
K_2HPO_4	0.954
$CaH_4(PO_4)_2H_2O$	0.540
Fe citrate	0.118
Ca lactate	1.300

the behavior of the animals was substantially the same. There was little growth in any case. Of the three additions described, the proper adjustment of the inorganic content is the most beneficial (rat 315–Chart 1).

Obviously more than a single dietary factor is at fault in determining the failure of young rats to grow on maize. We conducted a series of experiments in which maize was fed with two purified food additions: (e) maize, salts and casein, (f) maize,

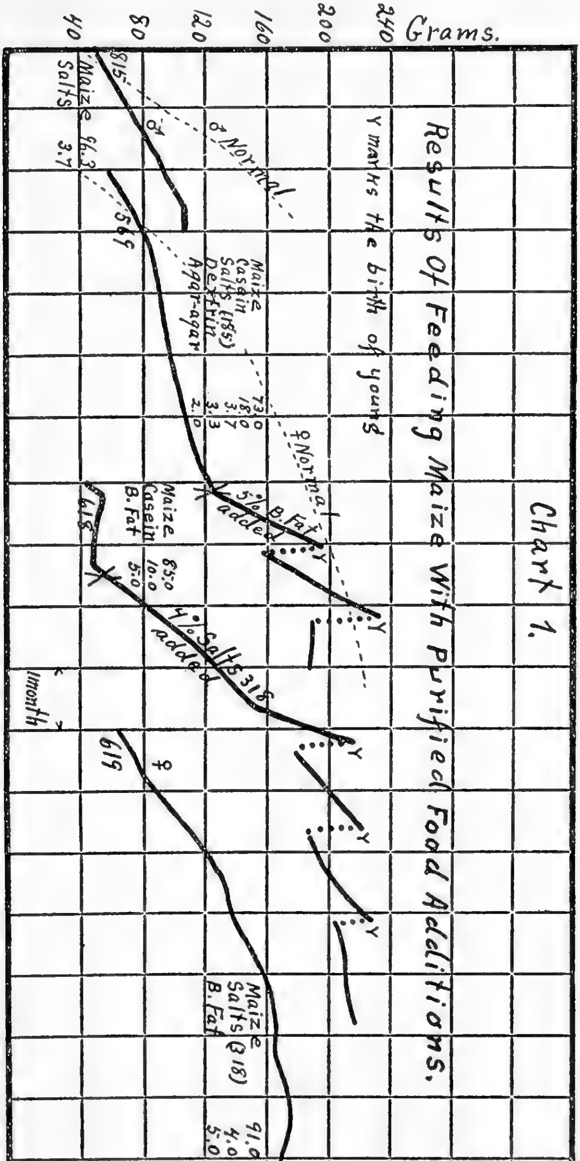
Chart 1.
Results Of Feeding Maize With Purified Food Additions.
Y marks the birth of young
Grams.
40
80
120
160
200
240
815 ♂
Maize 96.3
Salts 3.7
♂ Normal
569
Maize 73.0
Casein 18.0
Salts (185) 3.7
Dextrin 3.3
Agar-agar 2.0
♀ Normal
5% B.Fat added
Maize 85.0
Casein 10.0
B.Fat 5.0
618
4% Salts 318 added
1 month
619 ♀
Maize 91.0
Salts (318) 4.0
B.Fat 5.0

salts and butter fat, (g) maize, butter fat and casein. The results of feeding such diets are shown by the curves in Chart 1. Rat 569 made somewhat less than half the normal rate of growth over a period of five months when both the protein and inorganic content of maize were supplemented. The addition of butter fat at this time caused extremely rapid growth and during the following three months two litters of young were born, one, the second, being successfully reared. Rat 619 which received maize, salts and butter fat grew slower than the normal rate and showed decline after the age of six months. She never produced young. Rat 618 which was given maize, casein and butter fat, did not grow at all during a period of six weeks. Four per cent of a suitable salt mixture was then added, with the result that growth was promptly resumed and the animal reached the full adult size at the normal rate and produced three litters of young, all of which were successfully weaned. These records are each representative of a group of rats.

Chart 2 illustrates the behavior of rats with respect to growth and reproduction when fed wheat with two purified food additions,[16] casein and salts, rat 223; casein and butter fat, rat 380; butter fat and salts, rat 319. Rats in lot 223 in some instances grew rapidly to full adult size, but all were short lived. At the age of six months they were all emaciated and showed oedema of the eyelids with hemorrhage of the conjunctiva. In rat 380 which received wheat, casein and butter fat, growth was very slow. After being stunted during five months the addition of a suitable salt mixture induced a wonderful acceleration of growth and complete recovery from a condition of partial baldness, accompanied by a roughness of the skin and tail. Animals in this group have frequently suffered from a purulent bronchitis.

The stunted growth, lack of fertility and early decline which follows feeding wheat with salts and butter fat additions is illustrated by rat 319. When, however, wheat is supplemented with three purified additions, casein, salts and butter fat, it becomes capable of supporting growth, reproduction and rearing of the young in a manner which closely approaches the optimum, as is shown by the curve of rat 223 B.

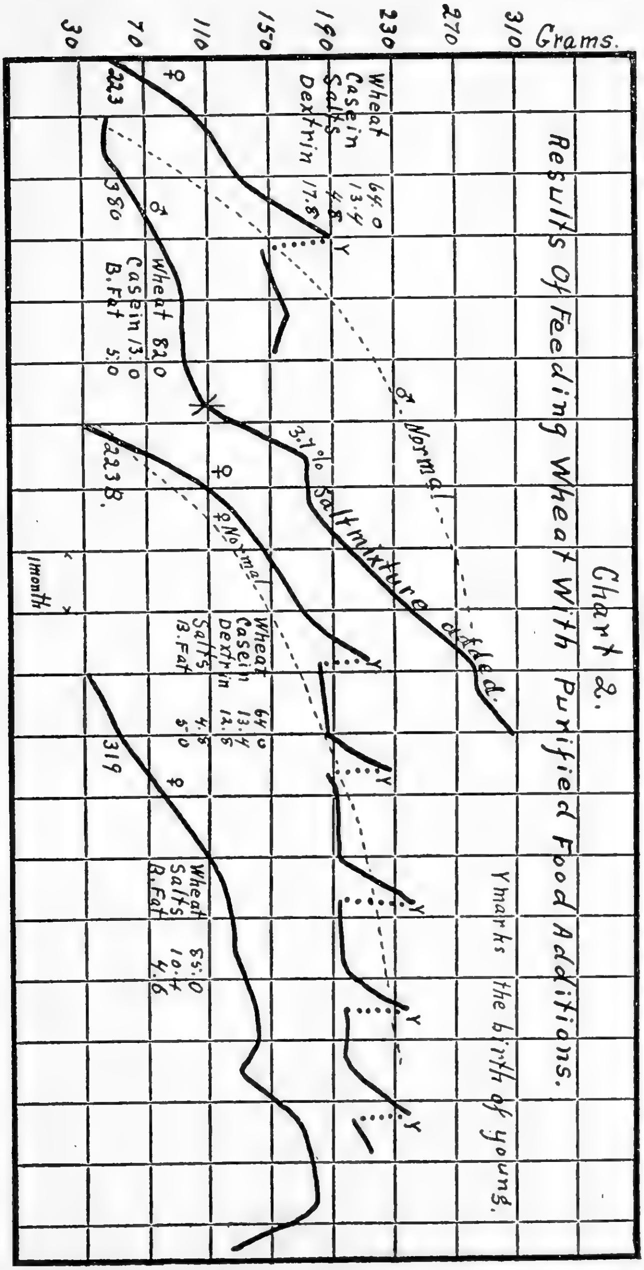
Chart 2.
Results Of Feeding Wheat With Purified Food Additions.
Y marks the birth of young.
Grams.
30
70
110
150
190
230
270
310
Wheat 64.0
Casein 13.4
Salts 4.8
Dextrin 17.8
223
♀
380
♂
Wheat 82.0
Casein 13.0
B.Fat 5.0
♂ Normal
3.7% Salt mixture added.
223B.
♀
♀ Normal
1 month
Wheat 64.0
Casein 13.4
Dextrin 12.8
Salts 4.8
B.Fat 5.0
319
♀
Wheat 85.0
Salts 10.4
B.Fat 4.6

Wheat and maize are therefore closely similar in their dietary properties. Each requires an improvement in its protein moiety, a suitable supplementing of its inorganic content and an added supply of the dietary factor which I have designated the fat soluble A. Each contains an abundance of the second unidentified dietary essential, the water soluble B, since without any addition of this substance excellent nutrition can be secured.

Let us consider for a moment the dietary properties of the oat kernel [17] as revealed by feeding it supplemented with single or multiple additions of purified foodstuffs. It becomes apparent from an inspection of Chart 3 that the same general relations hold as we have seen in the case of the maize and wheat kernel. The curves are each representative of the performance of a group of not less than four rats. Here, however, the addition of casein, salts and butter fat does not lead to normal nutrition. We have not yet been able to supplement oats with purified food ingredients and attain optimum results, where the oat kernel constitutes 70 to 80 per cent of the food mixture. We have not yet determined the cause, but it is evident that a high intake of oats over a long period causes injury to the rat. This is true also for the cow and I believe also for swine. Gelatin combined with oat proteins forms a much better protein mixture than does casein and oat proteins. This will be made apparent by an inspection of Chart 6, rat 647. (Compare Charts 6 and 3.)

The results of testing combinations of two or more kinds of seeds as a restricted diet has proven extremely interesting. The curves of four representative rats receiving diets made up on this plan are shown in Chart 4.[17] It proved a great surprise that we were not able to make combinations of the seeds of plants in any way which forms a ration capable of inducing normal growth in the rat, and an extensive experience has all tended to confirm our belief that this is true for swine and probably for other mammals as well. It is, however, possible to secure with rations restricted to seeds, nutrition of at least a fairly satisfactory character with pigeons [14] and possibly also with chickens. Chart 4 shows clearly the dietary factor which forms the most important cause in inhibiting the growth of the rat on these diets. It is the char-

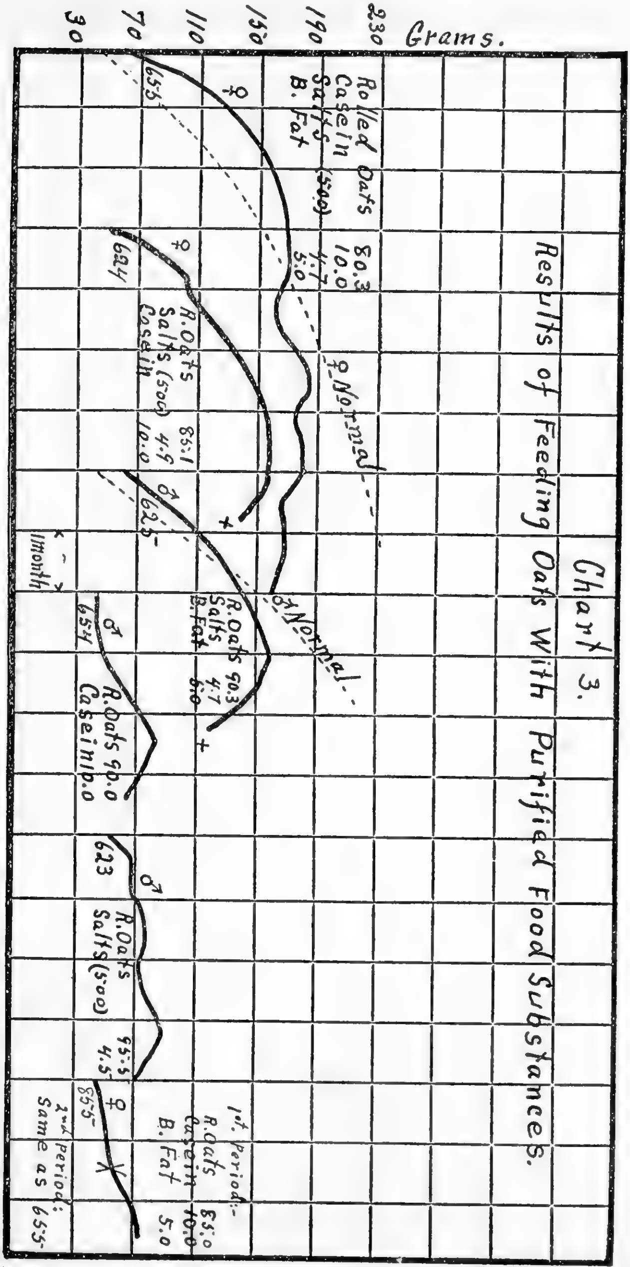
Chart 3.
Results of Feeding Oats With Purified Food Substances.
Grams.
30
70
110
150
190
230
Rolled Oats 80.3
Casein 10.0
Salts (500) 4.7
B. Fat 5.0
655
♀
♀ Normal
624
R. Oats 85.1
Salts (500) 4.9
Casein 10.0
♂
625
♂ Normal
R. Oats 90.3
Salts 4.7
B. Fat 5.0
1 month
654
R. Oats 90.0
Casein 10.0
623
R. Oats 95.5
Salts (500) 4.5
855
1st period:
R. Oats 85.0
Casein 10.0
B. Fat 5.0
2nd period:
Same as 655

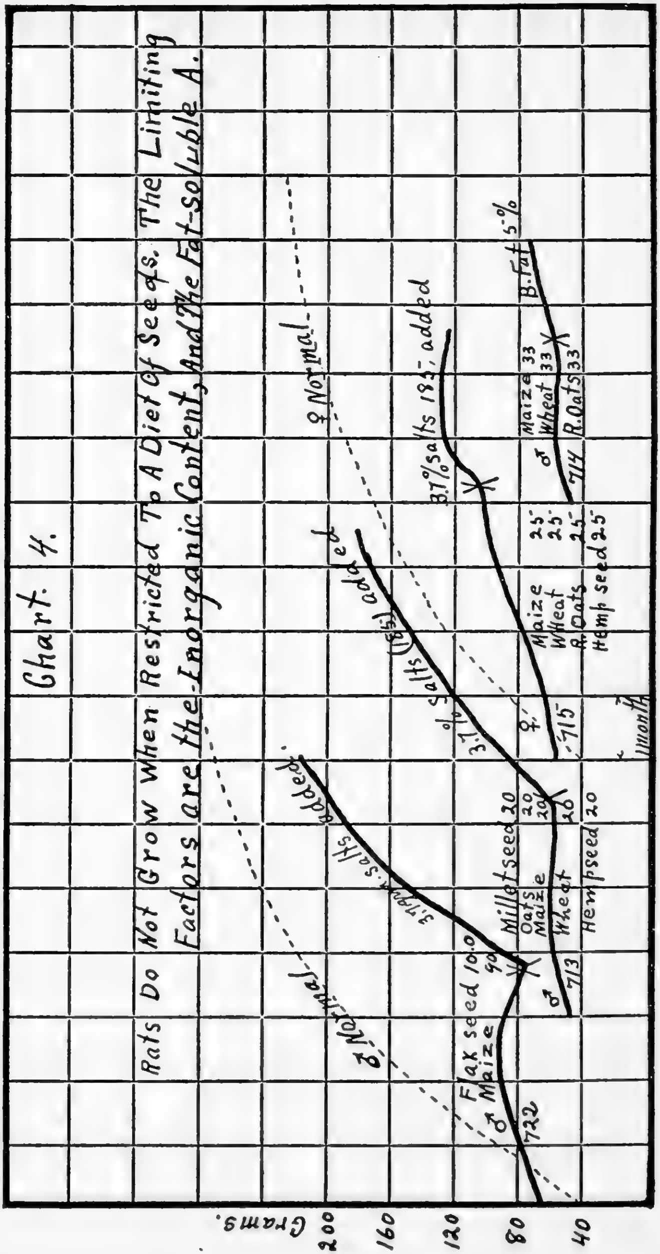
Chart 4.
Rats Do Not Grow When Restricted To A Diet Of Seeds. The Limiting
Factors are the Inorganic Content, And The Fat-soluble A.
♂ Normal
♀ Normal
3.7% salts added
3.7% salts (185) added
3.7% salts 185 added
Flax seed 10.0
Maize 90
Millet seed 20
Oats 20
Maize 20
Wheat 20
Hempseed 20
Maize 25
Wheat 25
R. Oats 25
Hemp seed 25
Maize 33
Wheat 33
R. Oats 33
B. Fat 5%
♂ 722
♂ 713
♀ 715
♂ 714
1 month
Grams.
200
160
120
80
40

acter and amount of the inorganic portion of the food mixture. When this is properly adjusted by means of salt additions growth can proceed on certain mixtures. After prolonged suspension on ration 713 the addition of salts led to growth. Equally pronounced failures with rations of this type containing beans or peas are abundant in our records.

In marked contrast to our failure to secure satisfactory nutrition with any of the many combinations of from two to five kinds of seeds, stand the nearly normal growth of rats which are limited from an early age to a monotonous mixture of a seed and a leaf. We have tested most thoroughly combinations of the alfalfa leaf with several seeds, since it can be secured as a meal and as a flour, the former being much employed in animal production. The products which we have employed were a very fine meal consisting principally of the leaves, but containing visible pieces of stem, and also a flour which was practically entirely derived from leaves. Both products came from immature plants dried in the sun.

Chart 5 illustrates the fact that rats can take from the age of 35 days a mixture of 60 per cent of any one of at least three seeds, oats, wheat, maize,[17] with 40 per cent of alfalfa leaves, and grow to a size closely approximating the normal, and produce young. The same proportions between polished rice and alfalfa,[14] peas and alfalfa,[17] and cottonseed flour and alfalfa [17] yield less satisfactory results, but with these growth is far superior to what we have ever observed with the seeds alone in any combinations or proportions, when fed to rats whose water supply was distilled, and therefore salt free.

On ration 685, oats and alfalfa,[17] 14 out of 17 young were reared. The one litter of five produced by rat 687 all died soon after birth. Four of the 7 young in the litter from rat 686 were reared. No young have been reared on the polished rice and alfalfa mixture (rat 478).

Two factors contribute toward making these combinations of seed and leaf superior to seeds alone. First, the character of the inorganic content of the leaf is much greater and is of an entirely different character from that of the seed. In the following table

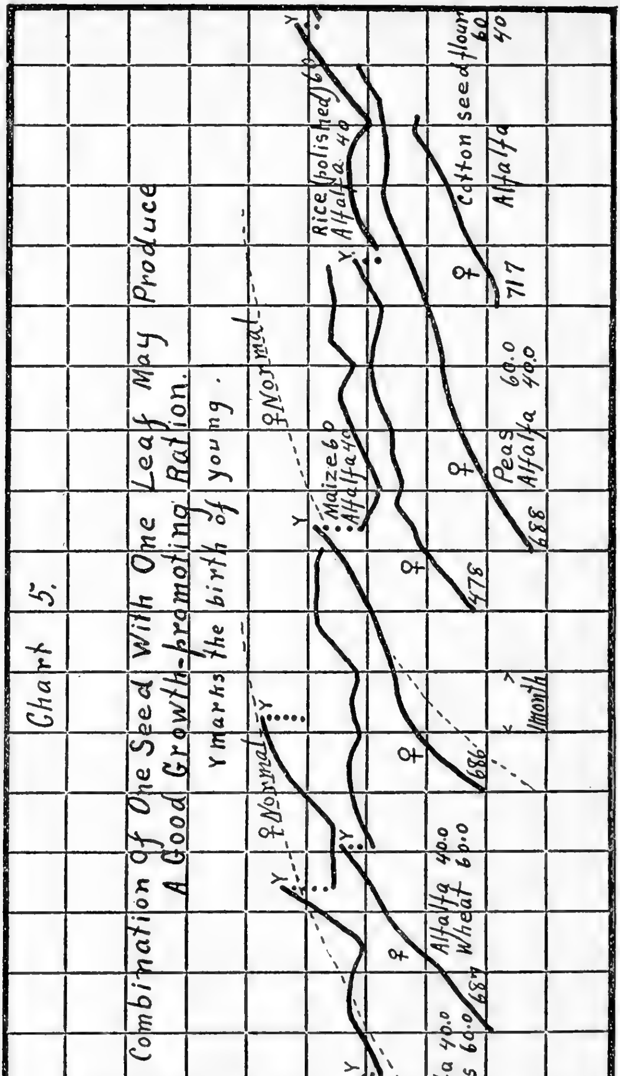
Chart 5.
A Combination of One Seed With One Leaf May Produce
A Good Growth-promoting Ration.
Y marks the birth of young.
♀ Normal
♀ Normal
Alfalfa 40.0
R. Oats 60.0
685
Alfalfa 40.0
Wheat 60.0
687
686
Maize 60
Alfalfa 40
478
Peas 60.0
Alfalfa 40.0
688
Rice (polished) 60
Alfalfa 40
Cotton seed flour 60
Alfalfa 40
717
1 month
Grams.
230
190
150
110
70
30

are tabulated the average analyses of alfalfa hay, cabbage, wheat, oat and corn kernel, potato and of milk.

In 100 Parts Dry Substance
(From Forbes Bul. 207, Ohio Exp. Station)

	Total Ash	K	Na	Ca	Mg	Cl	P	S
Alfalfa	7.380	1.641	0.097	2.146	0.223	0.298	0.338	0.172
Cabbage	10.850	2.510	.805	1.663	.234	.853	.711	.372
Wheat	2.080	0.504	.026	.041	.141	.058	.408	.009
Oat	1.830	.361	.058	.097	.086	.098	.385	.005
Corn	1.450	.359	.012	.023	.136	.013	.289	.005
Potato	3.79	1.890	.083	.072	.113	.131	.279	.099
Milk	5.67	1.160	.344	.909	.088	.791	.650	.057

It is fortunate indeed that the inorganic content of the seed and the leaf supplement each other in a manner which meets the demands of mammalian physiology. The second way in which the leaf supplements the grain is in respect to the as yet unidentified dietary factor, fat-soluble A. This substance is present in the alfalfa leaf to the extent of at least five times the amount in the maize kernel, so that any diet containing thirty per cent or more of this leaf is not enhanced by the inclusion of butter fat. Cabbage and clover leaves also are rich in this dietary essential, but our experience with these is less extensive. There is, of course, to be expected a supplementary relationship among the proteins of a mixture of two or more foodstuffs. It could hardly happen that the proteins from several sources would be particularly low in their content of the same amino acids. That the limiting amino acid is not the same in several of the more important seeds is made evident by the curves shown in Chart 6. Rats 652 and 493 received a similar food mixture except that the former had 10 per cent of gelatin supplementing wheat proteins in place of an equivalent amount of dextrin. The gelatin containing diet promoted growth at a rate greater than the normal expectation, as compared with a rate about half normal in the gelatin free control.[18]

The second pair of curves, rats 756 and 785 received rations alike in all respects, except that in the former the protein con-

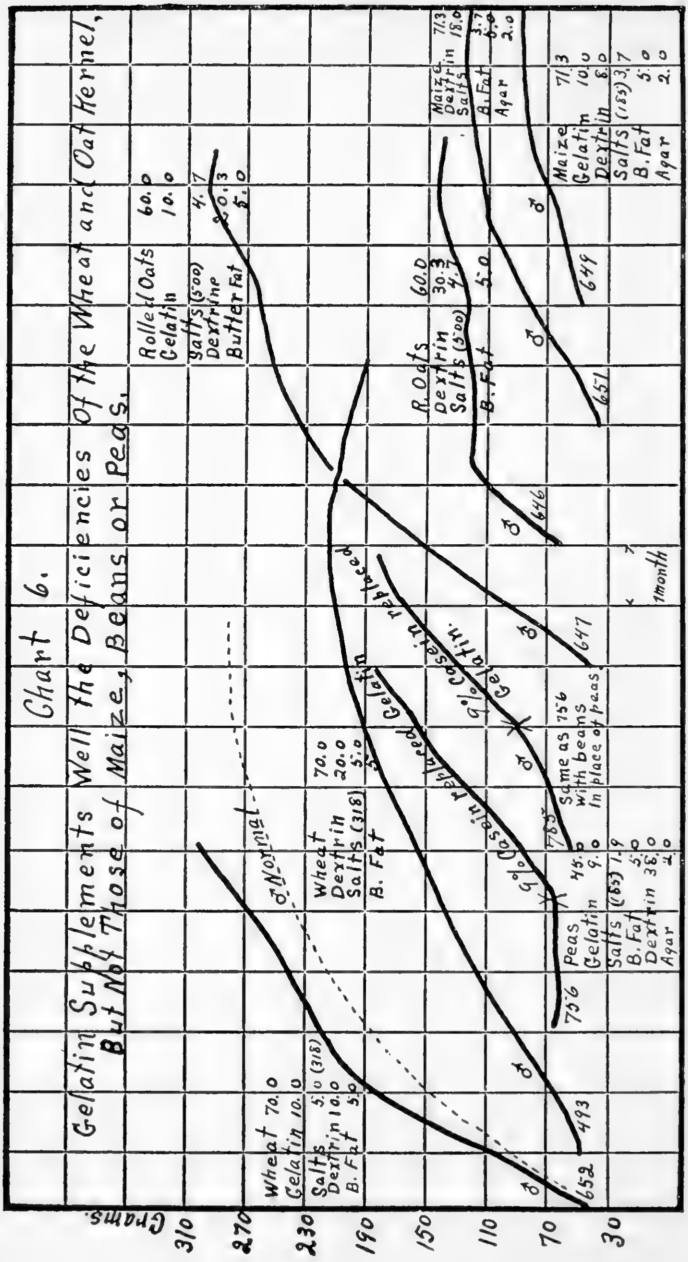
Chart 6.
Gelatin Supplements Well The Deficiencies Of The Wheat and Oat Kernel,
But Not Those of Maize, Beans or Peas.
Grams.
310
270
230
190
150
110
70
30
1 month
♂ Normal
Wheat 70.0
Gelatin 10.0
Salts 5.0 (318)
Dextrin 10.0
B. Fat 5.0
652
493
756
Peas 45.0
Gelatin 9.0
Salts (185) 1.9
B. Fat 5.0
Dextrin 38.0
Agar 2.0
Wheat 70.0
Dextrin 20.0
Salts (318) 5.0
B. Fat 5.0
785
Same as 756
with beans
In place of peas
9% Casein replaced Gelatin
9% Casein replaced Gelatin
647
646
Rolled Oats 60.0
Gelatin 10.0
Salts (500) 4.7
Dextrine 20.3
Butter Fat 5.0
R. Oats 60.0
Dextrin 30.3
Salts (500) 4.7
B. Fat 5.0
651
649
Maize 71.3
Dextrin 18.0
Salts 3.7
B. Fat 5.0
Agar 2.0
Maize 71.3
Gelatin 10.0
Dextrin 8.0
Salts (185) 3.7
B. Fat 5.0
Agar 2.0

tent (19.9 per cent) was derived solely from a mixture of peas and gelatin [17] and the latter from a mixture of navy beans and gelatin.[17] In neither case did the rations promote appreciable growth, although the entire group receiving beans grew noticeably better than did those getting peas. Gelatin does not supplement efficiently the proteins of either the pea or bean. In the second period in each case casein was substituted for the gelatin with the result that growth began at once at a fairly good rate. The proteins of the pea or the bean, when taken as the sole source of nitrogen, are of very low biological value.[17]

An inspection of the curves of rats 647 and 646 shows that gelatin has a very favorable supplementary relation to the oat proteins.[18] Gelatin when combined with the proteins of the maize kernel does not enhance the value of the latter.[18]

In 1914 we repeated the efforts of Slonaker [19] to find the effects of a strictly vegetarian diet of wide variety, on the growth of the rat. We offered 19 different foods of vegetable origin, giving the animals five or six at a time and changing their bill of fare at intervals of a few days. The list of foods included grains, leaves of plants, nuts, corn and wheat germ, peas, and flaxseed meal. None ever grew beyond half the adult size.[14] We duplicated in all essentials the results which Slonaker had observed. Having gained some insight into the peculiar dietary properties of certain natural foods I predicted two years ago that a mixture of maize 50, alfalfa leaves 30, and heated peas 20 would adequately nourish a young rat during growth. A trial proved that this strictly vegetarian food mixture induced satisfactory nutrition during growth, supported repeated reproduction and rearing of a fairly large percentage of young.[14] Chart 7 shows the curves from left to right of mother, daughter and granddaughter. A litter of great grandchildren were successfully weaned and appeared entirely normal when we discontinued the experiment. I should like to emphasize the importance of a proper adjustment of the well recognized dietary factors by pointing out that no young would be reared if this diet were changed to include less than 20 or more than 45 per cent of alfalfa, the maize replacing or being replaced by alfalfa in the adjustments.

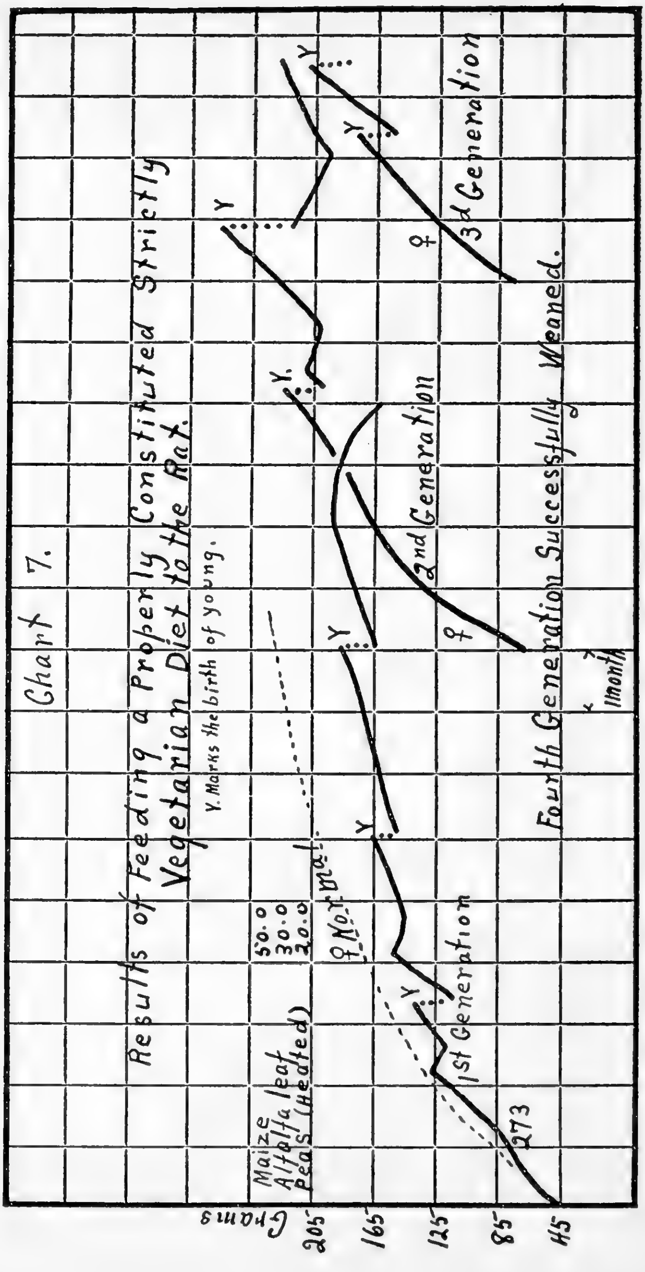
Chart 7.
Results of Feeding a Properly Constituted Strictly Vegetarian Diet to the Rat.
Y. Marks the birth of young.
Maize 50.0
Alfalfa leaf 30.0
Peas (Heated) 20.0
♀ Normal
1st Generation
♀ 2nd Generation
2nd Generation
♀ 3d Generation
3d Generation
Fourth Generation Successfully Weaned.
1 month
273
Grams
205
165
125
85
45

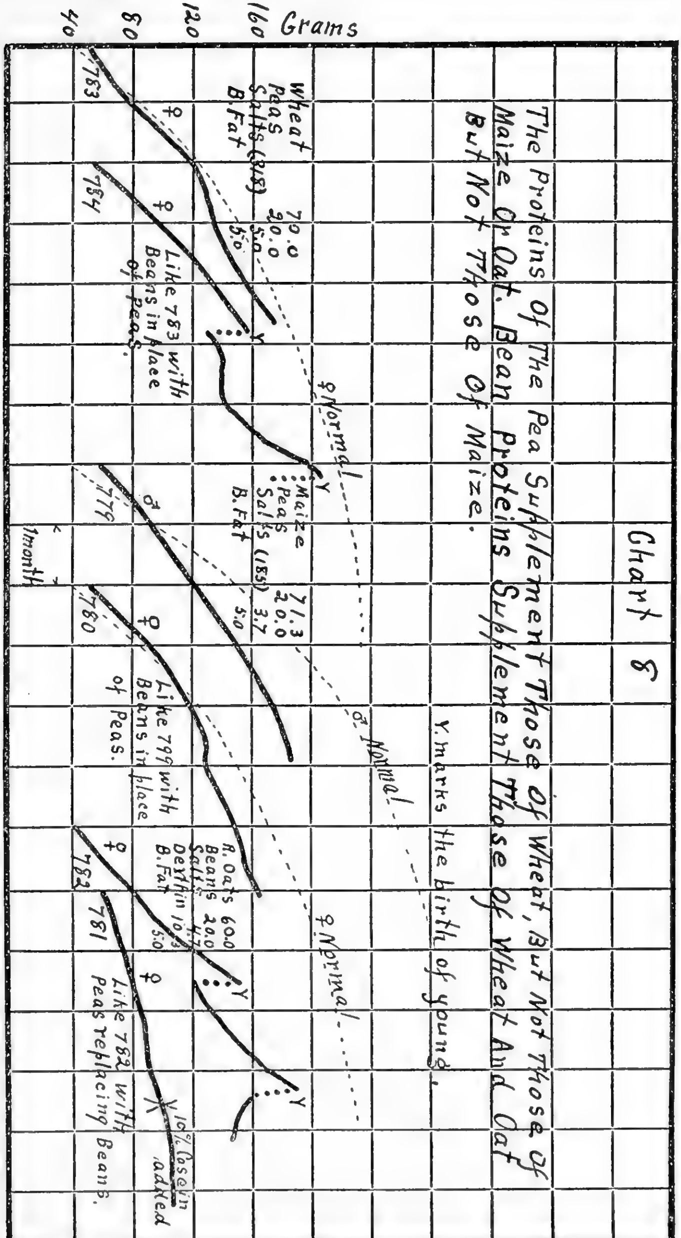
Chart 8
The Proteins Of The Pea Supplement Those Of Wheat, But Not Those Of Maize Or Oat. Bean Proteins Supplement Those Of Wheat And Oat But Not Those Of Maize.
Y. marks the birth of young.
Grams
40
80
120
160
783 ♀
Wheat 70.0
Peas 20.0
Salts (185) 5.0
B.Fat 5.0
784 ♀
Like 783 with Beans in place of Peas.
♀ Normal
779 ♂
Maize 71.3
Peas 20.0
Salts (185) 3.7
B.Fat 5.0
♂ Normal
1 month
780 ♀
Like 779 with Beans in place of Peas.
R. Oats 60.0
Beans 20.0
Salts 4.7
Dextrin 10.3
B.Fat 5.0
782 ♀
781 ♀
Like 782 with Peas replacing Beans.
10% Casein added
♀ Normal
Y

In Chart 8 are shown representative curves showing the failure of the proteins of either peas or navy beans to supplement very efficiently the proteins of the maize kernel;[17] rats 779 and 780. In either of these rations the peas or beans could be replaced by dextrin and a curve of nearly the same shape would be secured. Both pea and bean proteins improve the proteins of the wheat kernel,[17] for these curves are much better than would be secured if the legume seed were replaced by carbohydrate. (Chart 1–rat 619; Chart 2–rat 319.) The most remarkable result of studying these combinations is seen in the very different results of supplementing oat proteins with bean proteins as contrasted with peas. Pea proteins are wasted when combined with those of the oat (while the proteins of the navy bean and oat mutually make good each others deficiencies.[17] It follows therefore that the proteins of the pea and the bean are very dissimilar in respect to their yields of certain amino acids and that the same amino acid is not the limiting factor in each.

In Chart 9 are presented curves obtained with a series of diets designed to show the relative richness of the fat-soluble A in several natural foodstuffs. The diet, aside from its content of natural food, consisted of such a mixture of purified food materials as would support growth, when both the unidentified dietary factors, the fat soluble A and water-soluble B were added. The rôle of the natural food in these rations was to supply these two factors.[17] These curves make it clear that about 30 per cent of alfalfa leaf is the minimum amount which will supply the fat-soluble A in amount sufficient to induce complete growth and induce the production of a few young. The young were not reared on this ration. In Charts 1, 2 and 3 curves were shown which illustrate that even 70 per cent of corn, wheat or oat kernel do not furnish enough of the dietary A to support complete growth and repeated reproduction and well-being over the full span of life.

It is of particular interest therefore to compare the effects of flax seed and millet seed as sources of the fat-soluble A. Both of these are richer in this substance than are the cereal grains, and millet seed proves to be unique among the seeds we have

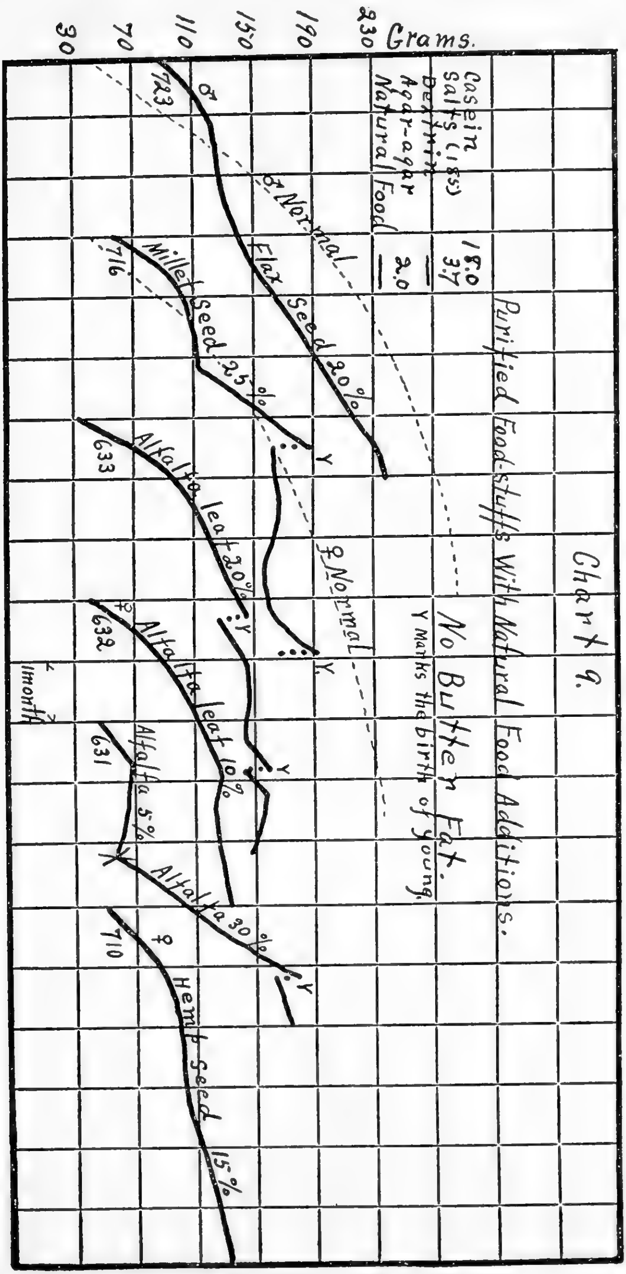

Chart 9.
Purified Food-stuffs With Natural Food Additions.
No Butter Fat.
Y Marks the birth of Young.
Grams.
30
70
110
150
190
230
Casein 18.0
salts (185) 3.7
Dextrin
Agar-agar 2.0
Natural Food
♂ Normal
♀ Normal
Flax Seed 20%
Millet Seed 25%
Alfalfa leaf 20%
Alfalfa leaf 10%
Alfalfa 5%
Alfalfa 30%
Hemp Seed 15%
723
716
633
632
631
710
1 month

examined as a good source of this dietary factor. When 25 per cent of millet seed was combined with purified foodstuffs growth was completed and one rat has produced two litters of young. After eight months on this diet she appears to be in perfect nutrition. The slow but long-continued growth on these rations whose content of both the unidentified dietary factors was solely derived from such small amounts of hemp seed, flax seed and millet seed shows that these stand intermediate between the cereal grains on the one hand and the plant leaf on the other as sources of the fat-soluble A.[17]

We have carried out a series of experiments designed to show the minimum amounts of several seeds which are necessary to supply enough of the water-soluble dietary essential for the completion of growth and the production of young. Chart 10 illustrates the results of this inquiry. The diet in all cases consisted of a purified food mixture, to which was added 5 per cent of butter fat to insure an adequate supply of the fat-soluble A. The sole requirement of the portion of natural food incorporated in the food mixture was to furnish the water-soluble B.

While 15 per cent of either maize or oat kernel fails to induce growth at the maximum rate 15 per cent of wheat not only enables the animals to complete their growth, but induces sufficiently good nutrition to lead to repeated reproduction. However, no young can be reared on either of the two lowest wheat rations here described. The factor which is responsible for this failure is the low content of the water-soluble B (Chart 2–rat 223 B). Peas and beans likewise are shown to be rich in this dietary essential, but they are both poor in the fat-soluble one.[17] The fact that rats 744 and 743 did not respond promptly with renewed growth upon the addition of an alcoholic extract of wheat germ containing additional water-soluble unknown is without significance. After a long period of stunting, response to improved diet is not always immediate. Diets deficient in one factor only and for this reason leading to suspension of growth do not all lower the vitality of the animals in the same degree. The extent of the debility determines whether they can respond promptly with growth when the diet is improved.

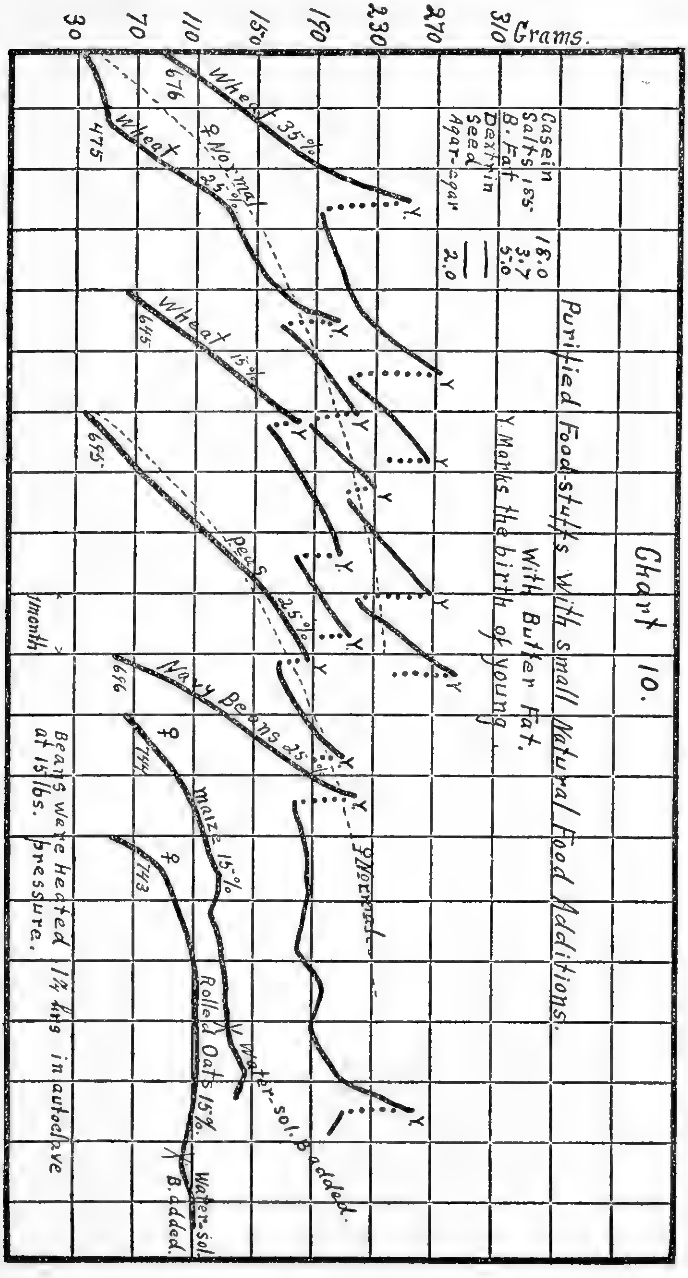
Chart 10.
Purified Food-stuffs with small Natural Food Additions.
With Butter Fat.
Y. Marks the birth of young.
Casein 18.0
Salts 185 3.7
B. Fat 5.0
Dextrin
Seed
Agar-agar 2.0
Grams.
30
70
110
150
190
230
270
310
Wheat 35%
Wheat 25%
♀ Normal
Wheat 15%
Peas 25%
Navy Beans 25%
Maize 15%
Rolled Oats 15%
Water-sol. B added.
Water-sol. B. added.
Beans were heated 1¼ hrs in autoclave at 15 lbs. pressure.
1 month

I desire next to attempt to offer an interpretation of certain experimental and clinical evidence which may seem to be out of harmony with the simple working hypothesis regarding the essential factors which are concerned in making a diet adequate, and with which I have attempted to explain the cause of the failure of animals to be adequately nourished on a considerable number of diets from various sources. I refer especially to the theory of Funk, which has found wide acceptance, that no less than four syndromes recognized as distinct pathological states, viz., beri-beri, scurvy, pellagra, and rickets, are all referable to inadequacy of the diet, and that each is the result of the lack of an adequate amount of a particular chemical substance in the food. To these Funk gave the general name vitamines. In seeking the causes of the inadequacy of certain diets I have dispensed with all but two unidentified substances.

There are two lines of evidence which offer strong support of the validity of the assumption that in the case of the factor which I have called the water-soluble B we are dealing with a single substance rather than with two or more. The first is based upon the results of feeding such rations as are described in Chart 10 in which all of this factor is supplied by as little as 15 per cent of wheat, or 25 per cent of peas or beans, alfalfa, etc. We have tested a list of foods which includes four grains,—wheat, oat, maize and millet, two legume seeds,—peas and beans, three varieties of leaf,—clover, alfalfa and cabbage, and three oil seeds,—hemp, flax and cotton seed, and find that in all these about 15 to 20 per cent is all that is essential with a mixture of purified foodstuffs and butter fat, to promote growth at the normal rate. While this does not establish the conclusion, we may reasonably expect that if the dietary factor B consisted of two or more chemical substances essential for normal metabolism, we should discover in some of these natural foodstuffs a shortage of one as compared with the other, in greater degree than is actually the case.

The second line of evidence which supports the idea that we are here dealing with but a single chemical substance rather than several, is to me still more convincing. It is as follows: A mixture

of purified food substances becomes capable of inducing vigorous growth when 3 per cent of wheat germ supplies the factor B and 5 per cent of butter fat the factor A. Now the wheat embryo can be completely extracted with ether without losing its potency in the least degree. The ether extracted residue, we have extracted eighteen hours in a continuous extractor with hot benzene in one instance and with hot acetone in another. The extracts obtained with benzene or acetone will not induce growth when combined with the purified food mixture containing butter fat, even when the extract equivalent to 28 per cent of wheat germ is added. In either case, however, 3 per cent of the ether-benzene or ether-acetone extracted residue is still as effective as ever. Now rats which are at all depleted with respect to the factor B do not recover when only 2 per cent of wheat germ is present in the ration. They do recover from a condition of extreme weakness, and partial paralysis on our ration with the extracted germ to the extent of but 3 per cent. While large doses of the material extracted from wheat germ by hot benzene or acetone contain enough of the factor B to cure a pigeon of polyneuritis, the amount of the curative substance extracted by these solvents is almost negligibly small.

The point I would emphasize as of significance in these results is the very small probability that if there were present two or more chemical substances, indispensable for the maintenance of physiological well-being, both or all should be practically completely insoluble in benzene and acetone as well as ether, and that both or all should be readily soluble in water and in alcohol. It would seem a remarkable coincidence if several compounds should show such complete uniformity in their solubilities, stability toward heat and so close a parallel in the proportions in which they occur in the seeds and leaves of widely different orders of plants. When contrasted with the very great variation in the content of the fat soluble essential in different seeds, and the richness of the leaf as compared with the seed in its content of this substance, it becomes the more improbable that in plant tissues generally there should exist a definite proportion between the remaining essential substances, whatever their number.

I am conscious of the question which you will ask concerning the cause of scurvy and of beri-beri. Are not these both dietary deficiency diseases? During the past two years Mr. Pitz and I [20] have repeated all the more important work reported by others on the production of experimental scurvy in the guinea pig by diet. We have confirmed the observations of Jackson and Moore,[23] to the effect that with a diet of oats and milk *ad libitum,* scurvy invariably results. Rolled oats induce the onset of the symptoms sooner than unhulled oats. Only those diets which produce feces of a character very readily eliminated will relieve or prevent the disease. Milk to the extent of 10 per cent of the solids of the diet supplies all the unidentified dietary factors necessary for growth in the rat or swine and probably for other mammals as well. We are convinced that the guinea pig suffers from scurvy on a diet of oats and milk because of the constipating character of the diet. Oats produce pasty feces, and the guinea pig, being unfortunate in the anatomy of its digestive tract, is quickly debilitated by its inability to empty the large and very delicate cecum. This harmonizes with our observation that orange juice, the panacea for scurvy in the human infant, gives protection to the guinea pig, but is not so efficient as to enable this species to take an oat and milk diet and grow continuously over a long period. We have furthermore been able to effect a complete cure of guinea pigs on a milk and oat diet after they could no longer walk, and showed badly swollen joints and the hemorrhage of the gums, by liberally dosing them with liquid petrolatum. Cavies so relieved have been as active and healthy as if on a diet of green grass, and have resumed growth and have continued to grow steadily, though at a rate slower than the normal rate during three months following the attack of scurvy, while confined to the same milk and oat diet which gave them the disease. The petroleum oil treatment was of course persevered in throughout this period. I question whether anyone would postulate the existence in liquid petrolatum of a "vitamine" specific as a protection against scurvy.

I venture to suggest that the failure of Holst [21] and his co-workers to prevent or relieve scurvy in guinea pigs by feeding

dried cabbage, finds its explanation in the failure of the latter to take up water in the intestine and again act as a succulent vegetable.

Hess[22] has recently, on the basis of anatomical lesions and for other reasons, suggested that scurvy and beri-beri show more points of similarity than have been recognized by clinicians and was inclined to consider the two as essentially the same pathological condition. His efforts toward curing scurvy with wheat germ, and with yeast were unsuccessful, whereas orange juice was potent as a curative agent, and he was forced to conclude that the two conditions were distinct. I will offer the suggestion that the latter view is correct. Scurvy in the guinea pig is the result of the retention of feces. I do not know whether or not the same is true of human scurvy. Neither do I know the cause of the hemorrhage of the joints and gums, whether they are the result of the absorption of a toxic substance of bacterial origin, which injures the blood vessels, or whether they are due to the invasion of the tissues by an organism, through an injured cecal wall. The recent observation of a streptococcus in the congested joints by Jackson and Moore, is suggestive in this connection.

I am inclined to attribute the protective power of orange juice as an antiscorbutic to its content of certain salts of citric acid, rather than to the presence of an unidentified organic substance of the class of the so-called vitamines. Its efficiency for the guinea pig appears to be somewhat less than for the human. This may well find its explanation in the much more delicate and inefficient structure of the digestive tract of the cavy as compared with that of the human, so that less efficient protective agents may serve for the latter than for the former. While guinea pigs are protected against scurvy by orange juice we have not seen them grow to any appreciable extent on a diet of oats and milk fortified with orange juice. If the results of experiments of three months duration are to be trusted orange juice does not appear to be any more efficient than is petroleum oil in protecting guinea pigs against scurvy when the animals are kept on a diet of oats and milk. I have dwelt thus long on the subject of scurvy in the hope that the tentative conclusions I have offered may help

in clarifying the interpretation of a most confusing mass of experimental data.

That beri-beri is a disease of dietary origin there is no doubt. That it can be relieved in the fowl by the extracts which contain the dietary factor B is equally certain. Much less definite statements can be made regarding the other diseases, rickets and pellagra, which Funk has attributed to lack of specific substances in the diet. The evidence as I understand it seems to point strongly toward diet as at least an important contributing factor. Assuming that they as well as beri-beri are of nutritional origin, I venture to state that they are the result of unsatisfactory adjustments among the well recognized constituents of the diet rather than to the lack of specific chemical substances. I have shown you how diets may be designed so as to induce faulty nutrition as the result of the shortage of each of the two unidentified dietary factors A and B; of the inadequacy of the protein content, because of an unsatisfactory yield of amino acids, and as the result of an unsatisfactory composition or inadequate amount of the inorganic content of the food mixture. I may add to these a fifth, viz., malnutrition as the result of the presence of toxic substances in the natural foodstuffs, particularly cotton seed and wheat products, and perhaps also a sixth cause due to mechanical injury to the digestive tract caused by foods producing feces of unfavorable character (*e. g.,* oats) and by constant distention of the alimentary tract as the result of excessive fermentation of foods such as beans and peas which contain a large amount of hemicelluloses.

I have attempted to show, so far as experimental data are available, the specific dietary properties of our natural foods, and how these may be so combined as to make good each other's deficiencies and shortcomings. In presenting this data it has also been emphasized how great is the need of more knowledge in this field. It is indeed remarkable how difficult it is to secure combinations of natural foods which will serve as monotonous diets over a long period and promote in an entirely satisfactory way the well-being of an animal. The consumption of a varied diet makes in some degree for safety, but will by no means insure

safety. It remains to be seen whether or not the animals which we have brought to a condition of nutritive disaster by a series of diets, each of which was faulty in respect to but a single dietary factor, as inorganic salts or protein, or the unknown A or B, will reveal to the histologist and pathologist lesions analogous to those seen in the states of human malnutrition presumably of dietary origin. The inadequate diets of man are probably never highly satisfactory with respect to all factors but one. Rather they are in some degree unsatisfactory with respect to two or three dietary factors simultaneously, and the probability that they will be so increases greatly as the food supply tends to be limited to the seeds of plants, and such manufactured products as are derived from the endosperm of seeds. The time will doubtless come within a few years when very specific advice can safely be given as to the best way to plan a variety of human dietaries, but a consciousness of the paucity of our knowledge concerning the peculiar supplementary relationships among our natural foodstuffs, forbids more than a very general statement as to the safest policy at the present time.

A diet in which the seeds of plants form the principal part will be made more safe by the judicious use of milk and of the leaves and probably also of the tubers of plants. These have peculiar dietary properties which the best chemical talent of today fails to recognize, but which are readily demonstrable by biological tests.

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THE INFLUENCE OF NON-SPECIFIC SUBSTANCES ON INFECTIONS

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THE therapeutic measures used in the treatment of infections may be divided into two general classes: first, those specific substances which are supposed to destroy the infecting organism or neutralize its toxins, and second, those which aim to strengthen the natural processes that normally bring about recovery.

Of the specific substances which either destroy the infecting organism or render toxic products innocuous, we have specific anti-sera, and certain chemo-therapeutic substances. Among the most effective anti-sera may be mentioned diphtheria antitoxin, tetanus antitoxin, anti-meningitis serum, and a serum for the treatment of one form of pneumonia. Many others have been prepared, but there is still some doubt as to their real value as therapeutic agents. Of the chemical substances which have a direct destructive action on the infecting organisms, quinin, salvarsan, and emetin are probably the most important.

Bacterial vaccines, autogenous and stock, have been extensively used in the treatment of infections with the hope that they might cause an increased production of immune substances. Curative vaccine treatment rests primarily on the assumption that under certain conditions the production of specific antibodies can be increased by the injection of the bacteria causing the infection.

Originally it was the belief that vaccines were indicated in those conditions in which the infecting organisms are localized and more or less encapsulated, and thus unable to stimulate the body to produce a sufficient number of immune bodies to bring about recovery. According to these views it will be seen that the use of vaccines as a curative measure was restricted to localized infections.

It is a debatable question whether the practical results support the hypothesis on which specific vaccine therapy is based, and it will be difficult to explain by this hypothesis the results now being obtained in the treatment of acute systemic infections. In these conditions the infecting organisms are widely distributed through the body and should furnish the stimulus needed to call forth an abundant supply of antibodies.

When we compare the number of infectious conditions which a physician is called on to treat with the number of specific therapeutic substances at his command, it will be seen that in the great majority of instances he is compelled to rely on the use of agents which merely serve to strengthen and to sustain his patient. Recovery in these cases depends almost entirely on the gradually increasing concentration or activation of the normal protective agencies of the body, and the final results depend upon the patient's ability to resist the infection until this stage is reached.

The work done in most of the experiments made to find curative agents for the various infections has evidently been based on the assumption that a substance must be found which will act directly on the infecting organism, or that will cause a mobilization of those specific immune bodies which we are accustomed to look on as the means nature uses to bring about recovery. And yet one finds a considerable accumulation of evidence, both from the laboratory and from the clinic, which definitely indicates that certain non-specific and as yet ill-defined factors have a large share in bringing about recovery from disease. While the emphasis placed on certain phases of immunology and specificity, more especially the fruitful antigen-antibody conception of Ehrlich, has been of inestimable value, I am inclined to believe that it has resulted in the neglect of certain perfectly obvious lines of approach to medical problems.

SPECIFIC VACCINES

That specific vaccines are effective in the treatment of acute general infections is shown by numerous reports. In 1893, Fränkel [1] treated 57 typhoid fever patients by subcutaneous and

intramuscular injections of typhoid bouillon cultures sterilized at 62° C. with excellent results. Petruschky,[2] in 1902, and Von Peskarola and Quadrone,[3] in 1908, obtained similar results. During the Balkan war of 1913, Petrovitch,[4] inoculated subcutaneously 460 typhoid fever patients with typhoid vaccines, with a mortality of 2.9 per cent. Of 220 patients not treated in this manner, 12.8 per cent died. Biedl [5] states that Wiel treated 14 children suffering from typhoid fever, without a single death, and that Von Variot treated 69 cases by this method with excellent results. It must not be forgotten, however, that the mortality among children affected with typhoid fever is usually low.

Göer [6] treated successfully nine cases of the same disease with a soluble albuminous product obtained from typhoid bacilli, and Krumbhaar and Richardson [7] observed favorable results in 77 cases treated subcutaneously with typhoid vaccines. These authors found in the literature records of 1800 cases of typhoid fever in which the patients were treated with subcutaneous injections of typhoid vaccines with favorable results.

During the past two or three years the methods of using vaccines in the treatment of acute general infections have undergone revolutionary changes. Ichikawa,[8] in particular, was instrumental in bringing about this change. This author gave intravenous injections of sensitized typhoid bacilli to 87 patients with typhoid fever. Immediate recovery, so far as fever and general toxic symptoms are concerned, followed the intravenous injection of one to two doses of the vaccines. Slight hemorrhages were observed in a few instances. Biedl [5] treated 21 patients with typhoid fever by the intravenous injection of typhoid vaccines prepared by various methods. Of these 21 cases, 2 patients died as a result of uncontrolable hemorrhages from the nose. Of the 19 remaining, 17 made immediate recoveries so far as temperature and toxic symptoms were concerned, one had a recurrence with an associated broncho-pneumonia and died, and one patient had a slight recurrence which subsided immediately after a second dose. In discussing the explanation of these results he states that they may be due to the action of protein split products, aided by the mobilization of immune bodies, as

was suggested by Ichikawa. Gay [9] treated typhoid fever patients with intravenous injections of sensitized typhoid bacilli and states that the course of the disease was favorably influenced in 66 per cent of the cases.

The above references show the results of the treatment of acute general infections by injections of specific vaccines. While the results may not have been entirely uniform in character, sufficient evidence is afforded to show that the older ideas concerning the principles on which vaccine therapy were based do not afford a satisfactory explanation.

NON-SPECIFIC

Matthes,[10] early in the development of tuberculin therapy, demonstrated that to all intents and purposes the reaction considered specific for tuberculin could be produced when deuteroalbumose was used, and he showed that whatever difference did occur could be explained by the fact that the tuberculin fraction contained certain toxic peptones in addition. Matthes, later, went even further, expressing the idea that fever in general was produced by protein split products, and he suggested the importance of proteolytic ferments in this connection, thus foreshadowing the work of Vaughn in this country and the later German workers in anaphylaxis.

Fränkel,[1] in 1893, was probably the first to demonstrate the value of typhoid vaccines in the treatment of typhoid fever, and his report was followed almost immediately by that of Rumpf,[11] who obtained similar favorable results with a vaccine composed of the bacillus pyocyaneus. The medical profession, however, could see no merit in a non-specific method of treating infections, therefore the entire subject was dropped for a considerable period. In this country, too, the controversy occasioned by the Schäfer vaccines is pertinent. Here was a biologic product, which, according to the observations of many competent observers, did at times produce striking results in a variety of infectious diseases; whether or not it was a safe remedial measure, or whether it had defects, is not pertinent in this connection. The very fact that it did certain things at times should have led to a

study of why such favorable changes were brought about. The fact that it was palpably non-specific, however, was sufficient to warrant the stamp of disapproval by the medical profession.

Within the last three years, however, this view has been gradually changing, and it is significant to note that Wright,[12] than whom, of course, no one man has stood out more emphatically for specific therapy, recently made the following statement in this connection: "All of those who have had much experience with vaccines will have seen cases where therapeutic effects, lying quite outside the range of the particular vaccine employed, and therefore, as we thought, not quite creditable to science, have been obtained with vaccine therapy."

Schmidt[13] is among those who have observed that following vaccine therapy of any kind the body becomes resistant to a variety of commoner infections. He also called attention to the relatively low "infection index," as he termed it, which is present in carcinoma and pregnancy, and refers to Rokytansky's ideas on the antagonism between carcinoma and tuberculosis.

Von Wagner[14] utilized a similar non-specific method when he obtained favorable results in patients with progressive paralysis treated with tuberculin. He had observed, as had others, that intercurrent infections frequently cause a remission of symptoms in this disease which sometimes lasts for a long period. This led him to experiment first with tuberculin alone, and later with a combined tuberculin-antiluetic treatment. He believed that he got better results with the combined treatment than with the antiluetic treatment alone. Pilez[15] observed similar good results with tuberculin, while Donath,[16] noting the same influence of intercurrent infections in paretics, suggests the formation of abscesses in these patients by injecting turpentine into the subcutaneous tissues.

It is not intended here to recommend the use of tuberculin in the treatment of paretics, because it hardly seems warranted, but to indicate the same general phenomenon of a therapeutic effect from a non-specific method.

It is in the domain of coagulation disturbances that therapy of this nature has received particular attention in the past. The

beneficial effects of subcutaneous serum injections in hemophila is well recognized, although here, too, the exact mechanism is unknown. Originally the slight leucocytosis was regarded as the potent factor, but for this there is no proof. Results have been obtained with homologous and heterologous serums, and with whole blood; but decisive results depend considerably on the dosage, and marked fluctuations in the coagulation time frequently occur following these injections. That such injections do not alter the disease process through supplying directly some deficiency, but rather through stimulating the elaboration of some substance before lacking, seems to be the conclusion reached by recent workers. This would agree with the stimulating effects observed by Esch, Busse and Weber following the injection of serum and whole blood subcutaneously in tuberculosis and in pernicious anemia.

The dermatologists have had similar results within the past three years in a variety of diseases, including some cases of psoriasis which have proved refractory to other methods of treatment. Recently Engman and McGarry [17] report favorable results in the treatment of lupus erythematosus with intravenous injections of typhoid bacilli.

The therapy of typhoid fever has so far been the chief avenue of approach to the problem. A number of vaccines have been elaborated and used subcutaneously during recent years for therapeutic purposes, and the results have been, in general, very encouraging. It was not, however, until such vaccines were used intravenously that the striking pictures of complete abortion of the disease were obtained.

Ichikawa,[8] who was among the first to use the intravenous method of administering vaccines in typhoid fever, observed that the results in patients suffering from paratyphoid fever who had been treated with typhoid vaccines were similar in every way to those noted in typhoid fever. Kraus [18] obtained similar results in the treatment of typhoid fever with the intravenous injections of vaccines composed of colon bacilli. He also treated eight patients with puerperal sepsis with excellent results. Kraus was so impressed with this form of treatment that he suggests its

use in scarlet fever, plague, septicemia, etc. In addition to vaccines prepared with cholera bacilli, typhoid bacilli and paratyphoid bacilli, Lüdke [19] also used deuteroalbumose. He treated 23 typhoid fever patients, and of these, 19 cases were favorably influenced. There was one death in this series. Reibmayr [20] obtained similar results with colon and cholera vaccines.

Hiss and Zinsser [21] were among the first to make use of non-specific substances in the treatment of acute infections. They used extracts of rabbits' leucocytes in the treatment of epidemic cerebrospinal meningitis, in pneumonia, and in staphylococcus infections, and believe that the course of these diseases was favorably influenced. Floyd and Lucas [22] used the leucocytic extracts in the treatment of 41 cases of pneumonia, and report that the mortality in their series was about half that obtained in the same institution with other forms of treatment.

Miller and Lusk,[23] during the summer of 1916, treated a series of patients with typhoid fever with intravenous injections of typhoid bacilli and with secondary proteoses. In this series, 20 per cent terminated by crisis, and 20 per cent by rapid lysis. They also treated a series of chronic, sub-acute and acute cases of arthritis with these preparations with excellent results, as relief was afforded in the majority of cases. The authors do not state the number of patients treated by each method, but conclude that both gave the same results. The same authors [24] recently reported a second series consisting of 85 patients with arthritis treated with typhoid vaccines. Forty-five of these cases were acute, four being gonorrheal. Twenty-nine of the acute cases recovered promptly after 1 to 4 injections, 8 showed marked improvement, 6 moderate improvement, and 2 were unimproved. Nine of these patients had recurrences. There were 12 sub-acute cases, 10 of which cleared up completely within 3 to 5 days, and 2 patients were greatly improved. There were two recurrences in this group, but the patients made complete recoveries on further treatment. Nineteen chronic cases were treated, 10 of which showed definite improvement.

Culver [25] reports a series of gonorrheal complications, especially arthritis, epididymitis and acute prostatitis, in which the

patients were treated with a variety of vaccines composed of gonococci, meningococci, colon bacilli, and with secondary proteoses. Twenty-eight of the 31 arthritic patients were either completely cured or manifested a decided improvement. The twelve patients with acute epididymitis presented complete freedom from pain after the first injection.

Ziembowski [26] has recently reported on the results obtained in the treatment of 100 patients with intramuscular injections of 5 c.c. of boiled milk. He states that excellent results were obtained by this means in the treatment of septic war wounds, in erysipelas, in tuberculous bone and joint diseases, and in three cases of actinomycosis.

Matthers, in a personal communication, states that he has used various types of vaccines and pure proteins in the treatment of typhoid fever, lobar pneumonia, scarlet fever and acute arthritis. He infers from his experiments that the therapeutic results obtained in erysipelas and lobar pneumonia do not justify the use of this plan of treatment. In the other diseases mentioned his results correspond favorably with those reported by other investigators.

Manier, Petersen and I have treated 13 cases of arthritis, of which 3 were acute, 3 sub-acute and 7 chronic. Of the acute cases, two were definite cases of acute rheumatic fever. One of these patients, a child of 12 years, with multiple swollen tender joints, fever, etc., cleared up entirely within 24 hours after a single injection of secondary proteoses, while the other, an adult female with multiple joint involvement, temperature, etc., was entirely relieved after eight injections except for some residual pain in one shoulder. The third acute case, with a gonorrheal joint, received four injections with only temporary subjective relief and with but slight objective change in the joint. The three sub-acute cases received an average of five injections, with complete relief of all symptoms and a return of the involved joints to normal in every respect.

In the seven cases of chronic arthritis there were varying degrees of disability, from slight stiffness in the milder cases up to complete ankylosis of the majority of the joints of the

body in the most severe cases. The injections given the patients varied in number from two to eighteen. The results were, complete relief in three, marked improvement in three and no noticeable change in one.

Four patients with gonorrheal epididymitis with acute swelling and tenderness of the epididymis, urethral discharge, etc., were treated. In every case of this series there was immediate improvement following the first injection, the epididymis becoming smaller and distinctly less tender. In each patient there was complete relief, as evidenced by the return of the epididymis to its normal size and consistency, together with the disappearance of the urethral discharge and of pus from the urine.

Two patients with erysipelas were treated. One was first seen on the second day of the disease, at which time the condition involved almost the entire face, and, in addition, the mucous membrane of the mouth and of the pharynx. The patient was markedly toxic, with a temperature ranging from 100° to 103° F., and a pulse rate of 140. He was given two doses of a secondary proteose solution on successive days, following which his temperature and pulse rate promptly settled to normal, and his local condition, both on the face and in the mouth, cleared up promptly. The second patient was also seen on the second day of the disease, and while he was not so seriously ill, the symptoms did not clear up until after four injections.

The amount of secondary proteoses given by us depends upon the apparent toxicity of the patient. We usually begin with 0.25 c.c. of a 1 per cent solution and increase the dose according to the reaction obtained. When it is remembered that Lüdke,[19] Miller and Lusk,[23] and Culver [25] gave as high as 2 c.c. of a 4 per cent solution, it will be seen that the amounts given by us are small. Subsequent experience, however, may demonstrate that the larger doses are advisable despite the more severe reactions, and particularly in arthritic cases, where the reactions appear to be associated with little or no danger. Our purpose has been to obtain some increase in temperature with a slight chill, and this reaction we can usually obtain at the first injection with 0.25 c.c. of a 1 per cent solution. Injections were given in

most instances every day. As individuals vary in their reactions, we believe it better to begin with the smaller dose and thus establish the tolerance of each patient before pushing the treatment. In no instance were there any alarming symptoms.

It is almost impossible to determine beforehand the degree of reaction which will follow these intravenous injections. Some authors believe that it depends on the severity of the disease, while others consider the concentration of immune bodies in the circulating blood more important. From a general survey of the work done, however, it appears that there is some other factor, as yet unknown, which plays an important part in determining the severity of the reaction.

Apart from the action of the vaccines, the protein split products, and the effect of both homologous and heterologous serums, observations are recorded by Smithlen,[27] Müller and Weiss,[28] and Saxl, Bruck and Kiralihyda [29] on the effect of intramuscular injections of milk, by Mitlander [30] on salt solution, and by numerous observers on the effects of dextrose solution, colloidal metals, distilled water, etc. It seems probable that the reaction and beneficial effects observed from these substances is based on a similar mechanism in all cases.

REACTION

Immediately following an injection by this method of treatment there is usually a reaction which is sometimes severe. As a rule, there is a chill from one-half to one hour following the injection, and this may last 15 to 45 minutes. With the chill there is an increase in temperature of from 1° to 4° F., followed several hours later by a progressive fall. Associated with the drop in temperature there is a general relaxation, profuse perspiration and a rapid subjective and objective improvement. The pulse may or may not be increased in frequency. The blood pressure in our own cases was not altered. Some authors report that many of their patients had headache, nausea, etc., and again others state that the symptoms following the injections are not sufficient to cause serious discomfort. These differences may depend on the dosage used.

Following the injection the leucocytes are decreased in number, at times as low as 2000 per cubic millimeter. This leucopenia is followed by a gradually developing leucocytosis which usually reaches its maximum in from five to seven hours. The leucocyte count has usually returned to normal within 24 to 30 hours.

Immediately following the injection in acute infections, such as typhoid fever, there may be a permanent return to normal temperature—termination by crisis; the temperature and general conditions may improve more slowly—termination by lysis; or, all the symptoms may return and the disease progress as usual, uninfluenced in any manner, though usually it pursues a milder course. The temperature frequently drops to subnormal and remains so for several days in those cases which terminate by crisis.

COMPLICATIONS AND CONTRA-INDICATIONS

The only serious results that have followed the use of this form of treatment occurred in typhoid fever patients, but as a large majority of the cases of acute general infections treated were patients with typhoid fever, it is important to learn more concerning the danger attached to treating this class of patients with non-specific substances.

Hemorrhage is the most serious complication reported in typhoid fever. Ichikawa,[8] who used sensitized typhoid vaccines in 87 cases, observed hemorrhages in a few patients, one to three days after inoculation, but the frequency with which these were observed was less than that noted among the uninoculated. R. Schmidt[13] advises against its use in patients who have already had hemorrhages, or who give histories of having been bleeders. According to this author, bronchial and pulmonary complications also contraindicate its use. Biedl[5] treated 84 typhoid fever patients with typhoid vaccines, and two of these died from uncontrollable hemorrhages from the nose. He also believes that previous hemorrhages contraindicate this form of treatment.

Typhoid fever appears to be the only disease in which hemor-

rhages have been observed following this method of treatment. Kraus [18] does not mention it as occurring in his cases of general sepsis; it was apparently not observed in any of the paratyphoid cases, and Miller and Lusk [23] do not mention its occurrence in their series of more than one hundred arthritic cases.

According to R. Schmidt,[31] protein substances injected intravenously or subcutaneously tend to decrease the coagulation time, and it is well known that certain of the lower protein cleavage products also inhibit coagulation. On the other hand, the subcutaneous and intravenous injection of homologous and heterologous serums has been a favorite procedure for some time in the treatment of hemophilia.

Ichikawa,[8] Miller and Lusk,[23] and others, advise against this method of treatment in patients with organic heart disease, and Ichikawa warns against its use in pregnancy. In the opinion of Lusk and Miller its use is also contraindicated in hypertension.

The severity of the reaction is an important factor in determining whether this form of treatment should be used in any particular instance. When we consider that the strength of the vaccines of specific and non-specific nature which have been used in the treatment of typhoid fever varied from 100,000,000 to 4,000,000,000 bacteria to the cubic centimeter, it will be seen that the danger to the patient is not so great as the severe reactions would indicate.

And now, how are we to explain the action of these non-specific substances? Can it be explained according to our present ideas of nature's method of bringing about recovery from infection? I believe it is doubtful if our present theories on immunity will enable us to explain the action of these non-specific substances. It might be well, however, to take up in greater detail some of the explanations which have been advanced.

SELECTIVE STIMULATION

It is now the general belief that the hematopoietic organs are the chief source of antibodies, and not the tissue cells in general. As a corollary of this idea concerning the source of

antibodies, it would be reasonable to suppose that any disturbance of the hematopoietic system might alter the antibody formation.

In view of these facts, it is possible that the various agents may act as stimulants of the hematopoietic tissue, thus suddenly flooding the body with immune substances, and thereby overcoming the infection. According to Wright, vaccine injections were supposed to be followed by a negative phase, at least so far as the opsonic power was concerned. Contrary to this generally accepted view, Bull [32] has recently shown that this does not hold true following the intravenous injection of a typhoid vaccine in immunized rabbits. Bull noticed that the antibodies were not diminished; on the contrary, they were rapidly increased following the injection. If this is the mechanism involved, it is important to bear in mind that the stimulus itself is not a specific factor, but that the hematopoietic system has been attuned to respond to a non-specific stimulus with the production of a specific substance.

Various investigators have stated that the results obtained with these non-specific substances are due to the mobilization of antibodies. Thus Müller and Weiss [28] thought this was the explanation of the results which they obtained in treating the complications of gonorrhea with gonorrheal vaccines, but serologic tests failed to confirm this view. Ichikawa [8] attributed his results to the mobilization of antibodies. Kraus [18] believed at first that the phenomenon was very similar to anaphylactic shock, but concludes that the lack of specificity contradicts this view. For the same reason he believes that the results obtained are not due to a mobilization of immune bodies. Lüdke [19] found that the agglutination value of the serum of typhoid patients treated with proteoses was not changed. Reibmayr,[20] also, found no changes in the agglutinins following the injections of typhoid vaccines.

In this respect the observations of Moreschi [33] are interesting. Moreschi noted the persistent absence of agglutinins in leukemic patients who suffered from superimposed typhoid and paratyphoid infections. The patient with typhoid fever recovered, while the one with the paratyphoid infection died. Immune

bodies could not be demonstrated in either case. The recovery of the typhoid patient indicates that agglutinins may not be essential.

Experiments which we conducted last spring caused us to believe that the results obtained in the use of non-specific substances in the treatment of infections, were due to the mobilization of immune bodies. Dunklin, who was working with us, found a marked increase in antibodies following the intravenous injection of proteoses in immunized animals. A similar increase in agglutinins was found in two cases of typhoid fever in which the patients had been treated with the same preparation of proteoses. We have not had the opportunity to make further tests in typhoid fever patients, but repeated experiments made during the past few months have failed to show an increase of antibodies in immunized animals following similar intravenous injections. The arthritic cases do not afford opportunities for this character of investigations, therefore we have been limited in our work to animal experiments. The observations of others, however, appear to show that the mobilization of antibodies must play a minor rôle in recovery from infection following the use of non-specific substances.

HYPERPYREXIA

It is a common clinical experience that in some diseases, among them sub-acute joint diseases, neuralgia, diabetes, pernicious anemia, certain dermatoses, sarcoma, etc., distinct beneficial results follow at times on some intercurrent febrile condition. May it not be, then, that these non-specific substances influence the course of the disease by producing a high temperature?

Rolly and Meltzer,[34] Lüdke,[35] and other investigators, have reached the conclusion that high temperatures (from 40° to 42° C.) artificially produced, have a favorable influence on an established infection. Heated animals were distinctly more resistant to daily injections of small quantities of bacteria, but no difference was noted when single large doses were given. They also found that agglutinins and bacteriolytic substances are produced more abundantly in animals which are kept overheated.

In his discussion of the influence of high temperatures on infection, Lüdke [35] suggests, first, the possibility of the infecting organism being killed by the heat, and second, that a more rapid and firmer combination of the antigen and immune bodies is caused at high temperatures.

Culver [25] describes an instance in which a patient suffering from both acute gonorrheal urethritis and malaria, made a complete recovery from the urethritis following chills and fever lasting four days. He also states that one rarely sees a gonorrheal infection coexisting with fever-producing diseases like pneumonia, typhoid fever and malaria. It is well known that gonococci are particularly susceptible to high temperatures, and therefore this factor may be of importance in gonococceal infections, but it is doubtful if similar importance can be ascribed to high temperatures in infections due to such organisms as streptococci, typhoid bacilli, etc.

Inasmuch as a very sharp febrile reaction almost invariably follows the intravenous injection of specific and non-specific substances, the importance of this phase of the subject cannot be overlooked. In one of our cases we observed a reaction temperature of 107° F. within thirty minutes after an intravenous proteose injection. This high temperature was unaccompanied by a change in the pulse rate of any moment, or other untoward symptoms.

LEUCOCYTOSIS

The importance of the leucocytic reaction has been emphasized by various authors. Gay [36] and his associates believe that recovery in typhoid fever following the intravenous injection of a modified typhoid vaccine is due to a specific leucocytosis. More recently, however, McWilliams [37] has observed that this hyperleucocytosis is apparently not specific to the degree indicated by the work of Gay and Claypole, and that even normal rabbits respond with a marked leucocytosis to the intravenous injection of typhoid vaccine. This coincides with our experience. We must keep in mind, too, the fact that in typhoid fever, particularly, the normal course of recovery is not marked by a leucocytosis. On this ground alone we might be justified in

seeking the influence of some other factor or factors in the recovery which follows intravenous therapy.

On the other hand, Lüdke [19] states that there was no leucocytosis in his series of typhoid fever patients who were treated with albumoses, and Reibmayr [20] makes a similar statement for his series of patients treated with typhoid vaccines. Most of those who believe that the leucocytosis is an important factor in recovery think chiefly of phagocytosis; other possibilities, however, must be considered.

Hiss and Zinsser [21] state that immunity is probably in a large degree cellular in character, not only in the sense of phagocytosis, but also in the neutralization of toxins. They conducted a series of experiments with this idea in mind, using the leucocytes of rabbits, and came to the conclusion that "leucocytic extracts have a distinct modifying and curative action on infections." They believe that the results are due to the neutralization of endotoxins. Opie [38] found that leucocytes injected into the pleural cavities of dogs in which a tuberculous pleurisy had previously been produced, tended to inhibit the development of the process, as those animals which received the leucocytes lived longer than the controls. Recently, Bail [39] has advanced evidence that supports this theory. He found that a strong anti-chlora serum would not neutralize the endotoxin obtained from cholera bacilli, but that the toxin was destroyed if it was first incubated with a fresh emulsion of leucocytes and the antiserum then added. In these experiments the leucocytes were removed by centrifuging before the serum was added. Control experiments demonstrated that the leucocytic emulsion alone did not destroy the toxin. Heating the leucocyte emulsion 30 minutes at 58° C. destroyed its antitoxic action.

Bull and I [40] showed that leucoprotease will destroy the toxic extracts of typhoid bacilli and meningococci, and it is not improbable that a similar explanation will apply to the results obtained by Bail.

It will be seen, then, that in considering the part leucocytosis may play in the recovery from disease, we must consider other factors in addition to phagocytosis.

MOBILIZATION OF FERMENTS

Petersen and I have already pointed out that in experimental animals intravenous injection of bacteria, [41] kaolin,[42] protein split products [43] and trypsin [44] is almost invariably followed by more or less marked mobilization of serum protease and usually of esterase.

Similar reactions occur in patients following the intravenous injection of vaccines and proteoses, but not to the same degree nor with the same regularity as in animals. In considering the possible effects of such a mobilization of ferments, both protease and esterase, we must keep in mind the fact that a variety of reactions may occur. The serum protease, as other tryptic ferments, is without effect on bacteria;[45] but if we consider the source of the intoxication which occurs in the diseased organism as primarily due to protein split products derived from the bacteria, then such a mobilization of protease may be of considerable importance in the process of detoxication, as the toxic fragments are hydrolized to lower and non-toxic forms. Petersen, Eggstein and I[46] have discussed this possibility in detail in its relation to pneumonia.

As a result of this action of the proteolytic ferments the diseased organisms would rid itself for the time being of the toxic substances in the circulating blood, although the disease process itself, and the infecting organisms, would possibly continue in existence, causing further injury. This would seem to be the explanation of the clinical picture obtained after intravenous therapy in those instances in which only an incomplete or transitory effect results. In the majority of these cases the patient presents a considerable improvement, both subjectively and objectively, on the day or days following the injection, despite the fact that the temperature may recur or even continue uninterruptedly. This hypothesis will not, however, explain the continued well being of the patients treated in the early stages of diseases such as typhoid fever. It is difficult to understand this unless it is due to cellular resistance acquired as a result of the injections.

The influence of the mobilized ester splitting ferments is as

yet obscure. In the ultimate analysis, of course, we must turn to ferments of this nature in order to dispose of the invading organism, because it is more than probable that the actual surface of the bacterium consists largely of lipoids, or intimate lipoprotein combinations, for the destruction of which esterase splitting ferments are probably essential.

Citron and Reicher,[47] Peritz,[48] and others, state that the serum esterase is increased in patients who suffer from infections due to lipoid rich organisms such as tubercle bacilli and lepra bacilli. They believe that a high esterase titer in these diseases indicates increased resistance on the part of the host.

ANTIFERMENTS

When discussing the increased resistance to infections said to be present in some conditions, among them carcinoma, pregnancy, and as a result of vaccine therapy, I called attention to the fact that during such states a relatively high antiferment index is a well recognized accompaniment. The question arises whether this is a mere coincidence, or whether there is some casual connection between the antiferment increase and the increased resistance to infection.

Petersen and I [49] have previously shown that the antiferment power of the serum depends on the amount of unsaturated lipoids present in a highly dispersed state in the serum. Consequently, any factor which will tend to increase these lipoids, either by increasing the supply or by decreasing the utilization, will increase the antiferment titer, while conversely, any influence decreasing these lipoids, their dispersion or their unsaturation, will tend to decrease the titer.

Wright [50] worked with saprophytic and serosaprophytic organisms, and noted that the addition of antiferment to the culture medium completely checked the growth of the serosaprophytic bacteria. He also found that even the saprophytic bacteria grew less luxuriantly when no proteolytic ferment was added. The direct influence of the antiferment cannot be as simple as Wright would assume, as Rettger, Berman and Sturges [51] showed that the ordinary pathogenic organisms do not derive their protein

requirements from native or even partly hydrolized proteins, but solely from the lowest split products. The antiferment inhibits only the action of tryptic and not the peptolytic and ereptic ferments. Of course, when we are dealing with a definite tryptogenic organism it becomes apparent that an increase in antiferment would offer an increased factor of resistance against its growth.

The immediate effect of the vaccine and proteose injections is not an increase, but a distinct decrease, in the antiferment titer for a short period of time, followed later by a rise. The exact cause of these changes in the antiferment index remains undetermined.

PHYSICAL CHANGES IN SERUM

We have recently found that there are distinct changes in the viscosity of the serum of animals undergoing immunization, and that similar changes occur in anaphylactic shock. This alteration may be of more than theoretical interest in our interpretation of the results obtained by the intravenous injections of non-specific substances.

Weil [52] and others have recently shown that antigen and antibody may coexist in the blood of the living animal, while Joachimoglu [53] has demonstrated that in anaphylactic shock the precipitins immediately disappear. The disappearance of precipitins, which no doubt is due to their combination with the antigen, would probably bring about conditions favorable to protease actvity, as Bulger [54] and others have shown that proteolysis in serums is active following the formation of precipitates. It is well to bear in mind the possibility that the changes in viscosity following the intravenous injection of non-specific substances may cause a combination of the antigen and antibody which is already present, and thus duplicate the conditions produced when additional antigen is introduced.

Similar changes may occur in the serum of patients with acute general infections when they are treated with non-specific substances. Under these conditions it is not difficult to understand how areas of lowered antiferment content would be obtained

in which proteolysis would occur. In such instances we may have a temporary increase in intoxication owing to the hydrolysis of the native bacterial proteins to toxic substances, and then a detoxication due to their further hydrolysis. This may be the explanation of the chill which is followed by a drop in temperature, that occurs after intravenous injections of various substances. These changes in viscosity may also help to explain the fall in antiferment strength which follows the injections.

As stated before, however, these serum changes are more or less temporary in character, and will therefore not explain the permanent recovery of patients treated in the early stages of such diseases as typhoid fever.

CONCLUSIONS

According to our present views the symptoms of an infection are the result of the struggle between the infecting bacteria and their toxins, and the protective agencies of the host. Theoretically, then, the results observed might be due either to the destruction of the infectious agent with its products, or to the fact that the cells of the host become resistant to the action of these agents, in either case, from our point of view, the disease ceases to exist. Theobald Smith,[55] in 1910, said: "The effectiveness of vaccines applied in the course of acute febrile diseases, such as typhoid and pneumonia, must be accounted for by principles of which experimental medicine has as yet no definite knowledge," and this view apparently holds true at present.

That the intravenous injection of non-specific substances exerts a marked influence on those infections in which it has been tried is very evident. It is not believed, of course, that these newer methods will cure all cases of infections. They do, however, open up new possibilities and suggest new methods for attacking infections of unknown etiology, as also those caused by organisms for which we have no specific antiserums. That all cases are not benefited does not necessarily reflect on the value of the treatment—there are very few theraputic measures which do not have the same objection.

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METABOLISM OF MOTHER AND OFF-SPRING BEFORE AND AFTER PARTURITION*

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IN presenting to the Harvey Society the results of several years' work on various phases of the physiology of reproduction, I wish first of all to make some acknowledgments. As I proceed you will observe that the researches in which I have participated represent but a small portion of the total body of information which I shall endeavor to interpret and, in making these acknowledgments, I hope I shall not seem to magnify the importance of my own work. I experience peculiar pleasure in making use of this public opportuntiy to acknowledge my indebtedness to my chief, Professor Graham Lusk, whose interest and encouragement have been never-failing. My thanks are also due to Dr. F. G. Benedict for the courtesies of his laboratory in doing what was, I believe, the most important piece of work in which I have participated; to Dr. J. Cliffton Edgar, head of the maternity service of the Cornell Division at Bellevue Hospital, and to Dr. L. E. LeFetra, head of the children's service there, for the facilities of their wards; and finally to Dr. T. M. Carpenter, Dr. H. C. Bailey, and Dr. B. R. Hoobler, who have been associated with me in authorship. These last named gentlemen have borne their full share of the work and, I trust, have had their share, also, of the pleasures resulting from the pursuit and acquisition of scientific data.

Because of the limitations of time imposed upon me, I shall be obliged to limit my remarks to three phases or chapters of the general subject: (1) the nutritive relations of mother and fetus; (2) the substance metabolism of the mother as modified by the presence of the fetus; and (3) the energy metabolism of mother and child both before and after parturition.

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I. NUTRITIVE RELATIONS OF MOTHER AND FETUS

Any adequate comprehension of the metabolic relationship between the mammalian mother and her offspring presupposes a broad view of the whole subject of reproduction. As in so many other departments of the physiology of man, interpretation of vital activities is constantly aided by reference to corresponding phenomena in lower organisms. I suppose we shall never understand fully what metabolism, nutrition, respiration, reproduction are—what they are in essence—as applied to our own tissues and bodies, until we understand their significance for the lower orders of life. We must have the viewpoint of the general physiologist and ofttimes of the naturalist.

Now the viewpoint of the naturalist regarding reproduction, since Weissmann's great work,[1] has been this: The germ cells or reproductive elements are not, strictly speaking, produced by the adult body; the adult body is produced and reproduced by the germ cells as a medium in which the specific stock can be perpetuated. Seen from this angle, for those forms in which the individual counts for little, reproduction becomes the whole end and aim of life. As Hatschek [2] frames the idea, "Fortpflanzung ist das Endziel der Labenstätigkeit." Continuing the thought, Hatschek says, "All the cells of the body stand at the service of the germ cells because in them is perpetuated their own being."

The student of reproduction in man only may easily lose sight of these broad, fundamental principles. It was for this reason, I think, that until a few years ago the mammalian ovum was regarded as nothing more than a maternal cell, just as much at the mercy of the maternal circulation as any other cell of the body; and the complicated provisions made after fertilization for insuring its supply of maternal blood were looked upon as beneficent, not to say providential, adaptations for the special care of the offspring. Partly through the cytological observations of Boveri,[3] Häcker,[4] Hegner,[5] and many others, who have demonstrated the independence of the *Keimbahn*, or germinal path, from one generation to the next in various lower forms of life (worms, crustacea, insects, etc.), and partly through the embryological studies of Hubrecht,[6] von Spee,[7] Peters,[8] Hitschmann and

Lindenthal,[9] Bryce and Teacher,[10] Herzog,[11] and Johnstone,[12] who have directed attention to the details of the process of implantation of the ovum in the wall of the uterus, it has gradually dawned upon us that the ovum never is a true body cell but is, to a large degree, an independent organism, capable even in the face of difficulties of looking out for its own nourishment at every step of its development. In fact, the only possible interpretation of events within the ovary previous to the liberation of the ovum is that in each follicle a certain cell is selected to grow and thrive at the expense of its fellows because (according to Miss Lane-Claypon,[13] quite fortuitously; according to John Beard,[14] by prearrangement) that particular cell possesses the complement of enzymes which enable it to appropriate the materials supplied by its less fortunate neighbors to its own purposes.

It is necessary to get this point of view—that events are from the start under control of the new organism rather than the old. Upon fertilization the processes of assimilation in the ovum receive a fresh impetus, the proteolytic and proteo-synthetic changes receive from the sperm cell an activating effect, something like the effect of a kinase upon a proenzyme, and the result is further growth at the expense of whatever materials the ovum comes in contact with. The follicle cells, which cling to the ovum when it is set free, and which, according to one view, prevent the ovum from becoming attached to the wall of the Fallopian tube, are digested away and, in some mammals at least, the tube supplies a nutritive secretion which is in all essential respects the analogue of the white of the hen's egg.[14a] Arriving at the uterus, the only reason why a fertilized ovum rather than an unfertilized one becomes attached to the wall, is that its cells (for there are many by this time) are hungry and they possess the means of satisfying their hunger.

From the histological studies made on an age series of hedgehog embryos by Hubrecht[6] and studies of the earliest human embryos by Bryce and Teacher,[10] Herzog,[11] and recently by Johnstone,[12] it is evident that the process of implantation from the standpoint of the embryo is simply a continuation of the

proteolysis by which it lays claim to its nutriment. The ectodermal cells which mediate this function are known collectively after Hubrecht as the *trophoblast,* or, a more exact term etymologically suggested by Minot, the *trophoderm.* Although an enzyme has not been demonstrated chemically in these earliest stages, it has been found by Gräfenburg as early as the second month in the human embryo and there can be no doubt, from the histological appearances of the earliest stages, that a very active one is at work or that it is produced by these trophodermic cells. Whatever they touch, according to von Spee, undergoes solution. Hence, wherever the ovum happens to come in contact with the uterine mucosa after the fringe of follicle cells has been digested and absorbed, it there adheres and soon dissolves a depression; the depression becomes a cavity and the cavity extends as the trophodermic cells increase in number. From the standpoint of the maternal organism, placentation, according to the interpretation first given by Sir William Turner and confirmed by most recent students [15] of the problem, represents a reaction designed to protect against the invader or, in modern phrase, to restrict the action of its enzymes. The large, specialized decidual cells are almost certainly active in the chemical defences of the maternal tissue.

By the time a circulation is needed to distribute the products of proteolysis to all the embryonic cells, a circulation is necessary also to connect the embryo with its advance lines of attack and we have the first steps in the formation of the true placenta. Stages described by Hitschmann and Lindenthal and by Johnstone, somewhat older than the Bryce and Teacher ovum, show clearly how the trophoderm of the primary villi become transformed into the double layer (syncytium and Langhans layer) of the definitive villi and how the trophoderm is responsible for the erosion and rupture of the maternal veins, thus establishing the intervillous circulation. "It is only this trophoblast," say Hitschmann and Lindenthal, "which is able to open up the vessels. The double layered villi no longer have this power; they serve mainly to extend the absorption surfaces." Hitschmann, in a later article, states that by the end of the third month in

human development "the villi have no further power of invasion of the blood vessels."

I must pause here to point out that menstruation is caused by an enzyme of very similar nature produced by the ovary just before the ovum is set free and acting upon these same blood vessels (Young[16]).

When the placenta is finished we have the following relationships: Maternal blood is separated from fetal blood by the two-layered trophoderm and by certain mesoblastic structures, making an arrangement practically identical with that of the wall of the intestinal villus. The question now arises, has in fact often arisen since the time of Harvey, whether the placenta acts, as Harvey expresses it, "by a sort of digestion," or in a purely mechanical manner. Time will not permit a full discussion of this question but I will cite some of the latest evidence and the considerations which must be taken into account in making a decision whether the fetus for a time surrenders control of its nutrition to the mother.

Truly diffusible substances, like glucose and urea, readily pass the placenta. Cohnstein and Zuntz[17] in 1884 showed that a hyperglycemia produced in the mother was followed by an increase in the sugar of the fetal blood. This has been placed on surer ground by recent methods in the work of Morriss of New Haven reported a few weeks ago in this Academy. Dr. Morriss[18] finds that the sugar in the fetal blood at the moment of birth is higher when the percentage in the maternal blood has been raised either by prolonged labor or by the use of anesthetics in delivery. Otherwise; *i.e.*, after easy labor unaccompanied by the use of anesthesia, the percentages on the two sides of the placenta are the same. It is probable, therefore, that glucose passes as readily through the placental barrier as it passes the intestinal wall. (We shall see later that these facts have their significance for the metabolism of the new-born). Studies on the glycogen of the placenta indicate, however, that there is some regulation of the amount of carbohydrate which is permitted to enter the fetal circulation. Both Chipman[19] and Lochhead and Cramer[20] have shown that up to the eighteenth day of gestation in the rabbit

(the entire period being twenty-eight days) glycogen is not found to any extent at all in the embryonic tissues, not even in the liver, but is found in abundance in the maternal side of the placenta. Goldmann [21] has shown the same to be true of the mouse but Driessen finds it not so strictly true of the human.[22] Contrary to the earlier teachings of Cl. Bernard and Pflüger, it appears that embryonic cells can not store glycogen [23] until they have reached a certain age. Glucose, arriving at the maternal placenta faster than it can be utilized by the fetus, is stored on the maternal side as glycogen until the last one-quarter of gestation when the fetal liver begins to assume its carbohydrate function, whereupon glycogen disappears gradually from the maternal placenta. Now it is scarcely open to doubt that the decidual cells perform this glycogenic function in obedience to some influence upon them by the fetus itself, for otherwise glycogen is not found in this situation.

Whether fat can be drawn from the mother's blood by the fetus raises some very interesting questions. Esterases [24] have been found in the placenta but no true fat cleavage has ever been proved to take place there. Ahlfeld,[25] Thiemich,[26] and Oshima,[27] all have failed to influence experimentally the percentage of fat in the fetal blood by feeding fat to the mother while S. H. and S. P. Gage,[28] likewise Mendel and Daniels,[29] failed to find stained fat in the embryo after feeding pregnant mothers with such materials. Dr. Slemons, in a personal communication, informs me that he has been studying the lipoids in maternal and fetal bloods for a year and has reached the conclusion that neither neutral fat nor cholesterol esters pass the placenta at all but that cholesterol does. Now cholesterol is a colloid in the blood and proof that it traverses the placental barrier is tantamount to proof that a selective activity is going on.[30] Bailey and I [31] have found that, while the percentage of total fat in the fetal blood is much lower than that of the maternal, there seems nevertheless to be some relation between the two and we are not sure but that there may prove to be some relation again to the severity of labor. This would not be surprising, in view of the fact that muscular work raises the fat in the blood, just as it does the sugar.

TABLE 1.—TOTAL FAT IN FETAL AND MATERNAL BLOODS AT MOMENT OF BIRTH (Murlin and Bailey, Unpublished).

	Arm Vein	Umbilical Vein
Case 1	0.60 per cent	0.22 per cent
Case 2	0.87 per cent	0.49 per cent
Case 3	0.72 per cent	0.48 per cent
Case 4	0.44 per cent	0.26 per cent
Case 5	....	0.24 per cent
Case 6	0.54 per cent	0.24 per cent
Case 7	0.49 per cent	0.18 per cent
Case 8	0.40 per cent	0.21 per cent
Case 9	0.36 per cent	0.14 per cent

There is scarcely any question about the presence of fat in the walls of the chorionic villi. This has been figured as stained by osmic acid and other reagents by many observers, especially Hofbauer [32] and Goldman. Hofbauer's figure shows fat in the deep layers of the syncytium in Langhan's layer and in the parenchyma of the villus, and since this is much the same histological picture as one gets in the absorption of fat by intestinal villi, where it is certain that fat is first split into its components and then resynthetized in the epithelial cells, Hofbauer interprets the picture as indicating the same chemical processes for the chorionic villi. The presence of fat in the villi, however, is not proof that it came through from the maternal blood. It may have been formed from carbohydrate or protein just where it is found. The fact that fat is found in abundance in the fetus at birth is amenable to the same interpretation; namely, that the fetal tissues have the capacity to form fat out of carbohydrate or protein. We have as yet, I should say, no positive proof that fat either is or is not taken up by the placenta. We can say with certainty only that it does not pass readily through the villi if it passes at all and it is never present in the same concentration in the fetal blood as in maternal. But this is not inconsistent with the vitalistic theory.

How proteins pass the placenta is the most important question of all. Gräfenburg [33] was not able to find evidence of proteolytic enzymes in the human placenta after the fourth month. Nevertheless, products of proteolysis, especially albumoses, have been found by Mathes,[34] Basso,[35] Hofbauer and others, both in

fresh placentæ and in minced placenta, after undergoing autolysis for a short time, at all stages of gestation. Hofbauer, although a firm believer in the vitalistic theory, was obliged to conclude that digestion stopped at the albumose stage and that synthesis takes place immediately the trophodermic cells are passed. That amino-acids can readily find their way through the placenta has now been definitely proved and Dr. Morse [36] in Slemons's laboratory has been studying the monamino-acid content of maternal and fetal bloods taken simultaneously at the moment of birth. He finds them nearly identical. Mr. Bock, in Dr. Benedict's

TABLE 2.—MONAMINO-ACID-NITROGEN IN FETAL AND MATERNAL BLOODS AT MOMENT OF BIRTH (Bock)

	Arm Vein	Umbilical Vein
Case 1	6.6 mgm. per 100 cc.	12.15 mgm. per 100 cc.
Case 2	8.9 mgm. per 100 cc.	14.92 mgm. per 100 cc.
Case 3	8.93 mgm. per 100 cc.	11.80 mgm. per 100 cc.
Case 4	7.44 mgm. per 100 cc.	9.57 mgm. per 100 cc.

laboratory at Cornell, at my request has made a number of examinations of the two bloods taken in the same manner and has found the percentage in fetal blood very distinctly higher than that of the maternal blood. These data are not yet complete enough for definite conclusions. Should it develop that the amino-acid content of the fetal blood is constantly, even though slightly, higher than the maternal, the conclusion would inevitably be (1) that these bodies are on their way out from fetus to mother or (2) are being produced in the placenta for use in the fetus or (3) again, that a selective activity is at work. Either of the last two interpretations would favor the idea that protein, the indispensable building material, continues to be modified by the placenta.

The transport of iron from mother to fetus can not be accounted for on mechanistic grounds. The mamalian ovum being practically devoid of yolk, contains no iron for the manufacture of respiratory pigment. There are two possible sources of this: The hemoglobin of the maternal blood, and certain conjugated proteins of the food. Concerning the transformations of the

latter as a source of iron for the fetus we know next to nothing. Many observations on the disintegration of red blood corpuscles by the trophodermic cells, however, make it certain that the main reliance of the fetus for this essential element is the maternal blood. Hemolysis produced by placental extract, reported by Veit and Scholten;[37] the eosin reaction of hemoglobin at the free border of the syncytium, seen by Bonnet; and the demonstration of loose organic compounds of iron in the deeper layers of the villi, by Chipman, Hofbauer[38] and Goldmann; may be regarded as pointing the way to a solution of this matter. In those animals which produce a "uterine milk" phagocytosis[39] on the part of the trophodermic cells probably accounts for the transfer of iron.

One is obliged to conclude from the weight of evidence at present that there is much more to be said for the vitalistic conception of the placental function than for the mechanistic. The very existence of the placental barrier, the fact that the two bloods can not intermingle would seem to imply the necessity of a process of naturalization at the border line of all materials which are used in the construction of the fetal tissues. Materials used only as a source of energy need not be so modified.

Under the mysterious guidance of the mechanism of heredity the proteins are built up into a new being which reproduces the essential characteristics of the phylum class, order, genus and species to which the germ cells, belong. Heape's[40] famous experiment in which the fertilized ova of one variety of rabbit were transferred to the uterus of a different variety and were born without showing any effect of the foster mother, and experiments by Castle[41] in which the same independence of the germ cells was demonstrated by transplantation of the ovaries of a black guinea-pig into a white female, leave no doubt upon this point—a human child is born human not so much because it is nourished by a human being as because the germ cells from which it came are human. Nobody knows yet how closely related germ cells and fostering mother must be in order that development may proceed. At all events, it is clear that the same necessity for enzymes to harmonize the building materials of the fetus to its own type

exists at every stage of development before birth, as after. The early enzymes with which the embryo starts out are just as much a part of the mechanism of heredity as are the enzymes of germinating seed. Indeed, one is tempted to assert that the mechanism of heredity is itself mainly an orderly succession of enzymes. Loeb,[42] Robertson,[43] Loeb and Chamberlain,[44] Riddle,[45] Goldschmidt,[46] and others have adduced evidence that the determiners of heredity behave like enzymes and Reichert,[47] from his study of homologous proteins and starches in different species and genera of animals and plants, has formulated a conception of the germ-plasm as "a complex physicochemic system of which an enzyme that starts the serial changes" and others that keep them going progressively are integral parts. A very significant observation by Abderhalden,[48] made just before the beginning of the war, has a direct bearing upon this question. Attempting to prove the synthetic action of ferments in the construction of proteins, Abderhalden made hundreds of different combinations of amino-acids and tissue extracts, but with no marked success until he tried the following: Digesting the several kinds of tissue, kidney, liver, thyroid gland, lung, etc., with pepsin, trypsin and erepsin until the digests were biuret-free, he added to the mixtures of amino-acids thus obtained a maceration juice extracted from the same tissues. Under aseptic precautions these mixtures were allowed to stand for five months at room temperature. At the end of this time there was clear evidence of synthesis but *only in those tubes which contained amino-acid mixture and extract* (*enzyme*) *from the same organ.* Kidney amino-acids were built up by kidney enzyme but not by thyroid; thyroid amino-acids by thyroid enzyme but not by kidney enzyme, etc. If this observation is confirmed we shall be obliged to infer that the repair in adult life and the development in embryonic life of each tissue protein is under the control of a specific enzyme, acting upon a specific substrate. It will be in order, then, to attempt to trace the different enzymes back step by step to the germ cells with the hope there to identify them with the chromosomes which are known on morphological grounds to contain the determiners of heredity. A substrate common to all tis-

sues after the earliest stages could be found only in the blood proteins. Seen with the eyes of the general physiologist, the nutritive relations of mother and fetus, then, find their explanation in the specificity of the proteins and the specificity of enzymes which lie at the basis of heredity—the reproduction of kind.

II. SUBSTANCE METABOLISM OF PREGNANCY

Stated in terms of the different combinations of protein building stones, or "stereoisomers," necessary to set up a new human organism, complete in all anatomical details, the requirements for fetal growth are enormous. Can the mother supply all of the building materials from her food, or must she perforce supply some structural elements, chemically speaking, from her own body? In some lower orders of animals the young, before being hatched or setting out upon an independent existence, consume the maternal body, the individual thus being sacrificed for the good of the species. Is gestation in the mammals in this sense to any degree "a sacrifice of the individual for the good of the species?"

Stated in quantitative terms, the substance requirements of the fetus are not large. According to Sommerfield,[49] a normal infant at birth weighing 4.340 grams contained just short of 100 grams of nitrogen, not more than would be contained in its mother's diet for ten days at most. Michel [50] has shown also the composition of the fetus at different stages of development for nitrogen, phosphorus, calcium and magnesium. It is noteworthy, according to these results, that up to the end of the seventh lunar month not more than one-fourth of prenatal growth has taken place.*

TABLE 3.—COMPOSITION OF THE FETUS (Michel)

Age weeks	N. grams	P. grams	Ca. grams	Mg. gram
16	2.941	0.662	0.419	0.021
20	6.054	1.448	2.214	0.077
24	11.048	2.444	4.082	0.133
28	16.005	3.527	5.881	0.190
40	72.700	18.673	33.260	0.815

* It would have been somewhat more convincing if Michel had used measurements instead of ages.

Suppose it were possible from the moment of conception to keep a balance sheet of these substances for the mother, setting her intake as food over against the output through her excretory channels. We should then be able to say whether a sufficient quantity of each substance had been retained to cover the requirements or whether the pregnancy resulted in a net loss.

Experiments of this character, with reference to nitrogen, carried out on animals by Reprew,[51] Ver Ecke,[52] Hagemann,[53] Jägerroos,[54] Bar and Daunay,[55] Murlin,[56] and Gammeltoft,[57] have developed the following facts:

1. Upon an adequate diet a dog, rabbit or goat may retain more than sufficient nitrogen to counterbalance the loss at parturition, plus the quota taken up by the uterus and mammary glands.

2. Upon a diet which is only sufficient to maintain nitrogen equilibrium in the non-pregnant condition, due allowance being made for difference in weight, the pregnancy will result in a net loss. The katabolic effect of the presence of the fetus is greater than the anabolic effect, taking the pregnancy as a whole.

3. While in the latter half of pregnancy there is always a plus balance, in nearly every instance recorded in animals (including the dog, rabbit, rat and goat), whatever the diet, there is in the first half either an actual negative balance or a strong tendency thereto.

The latter point may detain us for a moment. Here apparently is something quite unusual. One might reasonably expect that the moment conception occurs, retention of materials for the growth of its product would begin and, since the total quantity needed for development to the middle of pregnancy could be taken by the mother in a single meal, the retention of this amount spread over so long a time should be an easy matter. Katabolism, however, has the upper hand and Gammeltoft has shown that it is not possible to overcome the tendency by heavier feeding. Indeed, Jägerroos, Bar and I have each noted that the dog at about the third and fourth week of pregnancy, corresponding to the third and fourth calendar months in human pregnancy, may show lack of appetite and may even vomit. Bar

calls especial attention to the correspondence of this period to the period of morning sickness or the so-called *physiological vomiting* in women. It is true that a period of negative balance has not been seen in the woman. However, it is very significant that in the only two cases in which a nitrogen balance has been kept as early as the third month (one by Landsburg [58] and one by Wilson [59] the plus balance should be distinctly less in this month than in the second or fourth although there were no gastrointestinal symptoms. The only reason then must have been increased katabolism.

What is the explanation of this greater katabolism? It can be found, I believe, only in the nature of the means employed by the fetus for its nutrition at this stage. Recall the activity of the trophoderm up to the end of the third month in the human, and the fact that a proteolytic enzyme is demonstrable up to the end of the fourth month, a time when the placenta is considered to be completed in all essential structures. The time at which the negative balance gives way to a positive balance in the majority of the dogs studied corresponds well with the time at which, according to Bonnet,[60] the placenta in this animal is completed (thirtieth day). During the period of negative balance or tendency thereto, when the mother is losing more nitrogen from her body, the trophodermic cells on behalf of the fetus are producing the enzymes which enable the villi to invade the maternal tissues and become securely implanted in the decidua. When this invasion process has come to an end, nitrogen retention is easier. The facts clearly suggest that the extraordinary excretion of nitrogenous bodies is an inevitable wastage incident to indiscriminate action of enzymes and is closely comparable at this stage with the wastage incident to cancer. The proteolytic action of the trophoderm is more or less unrestricted; its enzymes are not yet confined to a definite locality; they may even be distributed throughout the mother's body.

This hypothesis, which was offered seven years ago [56] as an explanation of the negative balance in dogs and of the period of physiological vomiting in women, has been reviewed very favorably by Gammeltoft, but he criticizes it as laying upon the mother

the blame for a state of affairs which proves to be toxic for herself. Gammeltoft thus has overlooked the overwhelming evidence that the fetus, the neoplasm if one will, is producing the enzymes for its own use regardless of its effect on the mother. If the mother makes a prompt reaction, limiting the invader by means of the placenta, and possibly counteracting the enzymes with an antiferment produced by the specialized decidual cells, the period of heightened katabolism may be of short duration and of no serious consequence. Products of proteolysis may well be the cause of vomiting. Failure of the maternal reaction for defense and continued intoxication with such products are, not improbably, the cause of hyperemesis, or pernicious vomiting. Young's [61] explanation of eclampsia as due to infarction of the placenta with consequent autolysis and intoxication of the mother with toxins of protein nature, is readily accommodated to this view and would account for the high undetermined nitrogen of the urine of that disease first noted by Ewing and Wolf [62] and confirmed recently by Lossee and Van Slyke.[63] The rapid autolysis which Young demonstrates in such placentæ could be explained only by very active enzymes already present. The toxemias of pregnancy, then, it is suggested, may be explained as perversions of the chemism underlying the nutritive relations of mother and fetus.

When the net result of normal pregnancy is counted up for the woman, as has been done from the seventeenth week to the end of gestation for one case by Hoffström,[64] and from the nineteenth and twenty-fourth weeks, respectively, to the end for two others by Wilson, we find that the total retention of nitrogen exceeds the requirement of the fetus, uterus, placenta, membranes and mammaries by a very handsome amount.*

This accords with the experience of a large percentage of mothers who find themselves physically much better off at the end of gestation than at the beginning. Such advantage to the

* The human female enjoys a great advantage over the female of many other species in the fact that her offspring rarely weighs over eight per cent of her own body weight, whereas the dog, rabbit and many others deliver as much as twenty to twenty-five per cent of the body weight.

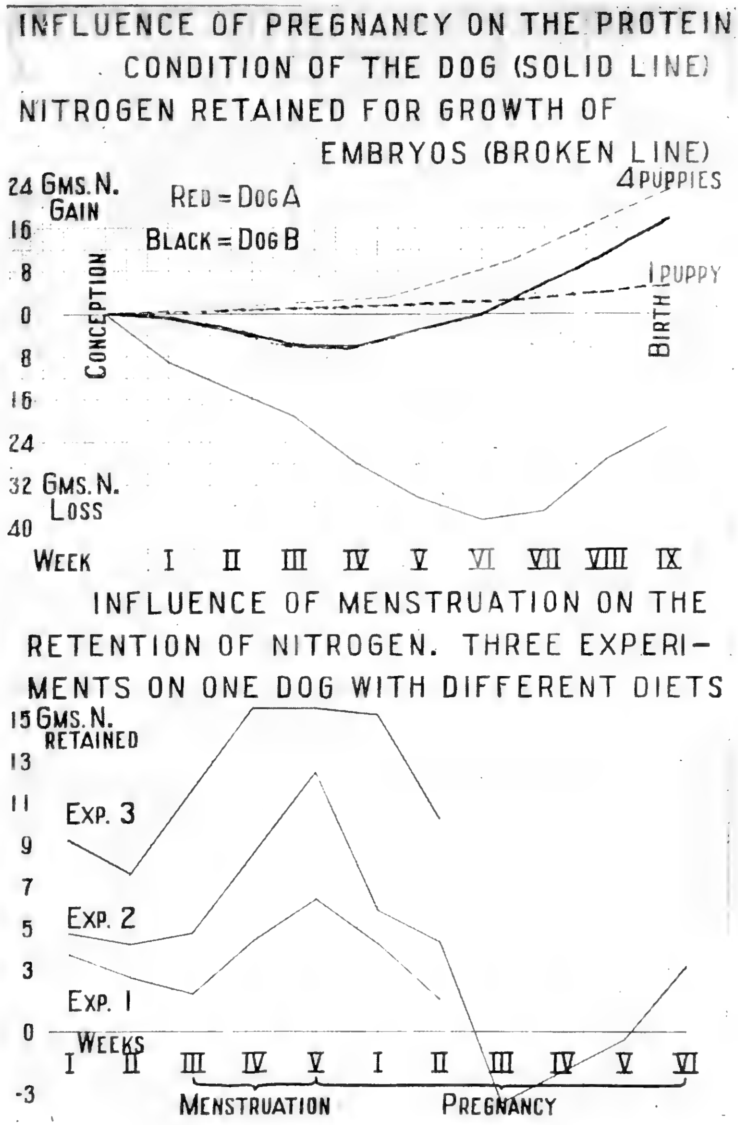
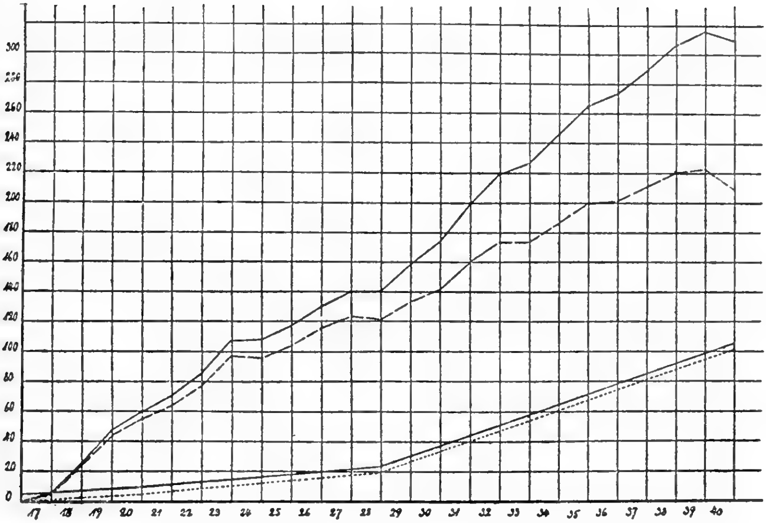


FIG. 1. The lower curves show the course of the total nitrogen retention during the menstruation and early pregnancy of the dog. Note the minus nitrogen balance in the third and fourth weeks of pregnancy. Upper curves show the total nitrogen gain or loss at each week of the pregnancy in two different dogs. Dog B finished with a minus balance (some 20 grammes behind) in a gestation with four pups. Dog A finished with about the same amount ahead in a gestation with one pup.

Fig. 2



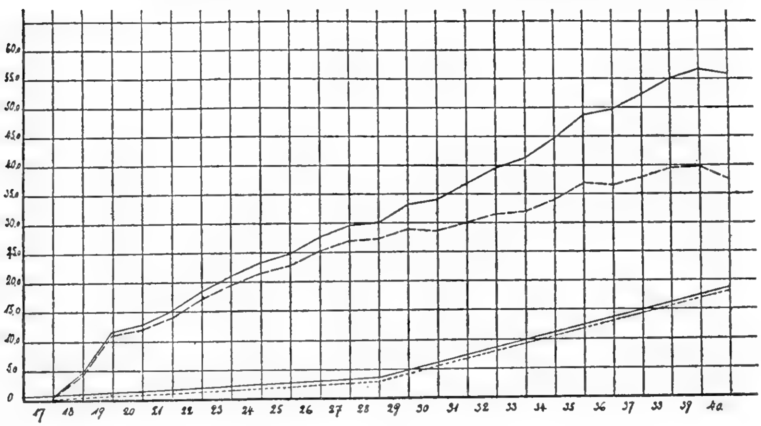


Fig. 3

Fig. 2.—Chart showing retention of nitrogen by Hoffström's case from the seventeenth to the fortieth week of pregnancy. ———, total N retained; - - - - -, N retained by fetus (based on Michel's analysis, table 3); — — —, N retained by mother's own body.

Fig. 3.—Chart showing retention of phosphorus by Hoffström's case from the seventeenth to the fortieth week of pregnancy. ——— total P retained; - - - - -, P retained by the fetus (based on Michel's analysis, table 3); — — —, P retained by mother's own body.

mother is, no doubt, a purposive one, as both Hoffström and Wilson suggest, in anticipation of the demands of labor and the lactation period. The pelvic and abdominal muscles appear to claim a large share of the surplus, but the gain is also more or less general. I have calculated the quantity of milk at 1.5 per cent of protein which might be produced from the nitrogen gained during the pregnancy for the three cases mentioned:

	Grams Retained	Equivalent in Milk
Hoffström's case	209.0	87 liters
Wilson's case II	284.5	114 liters
Wilson's case III	210.9	88 liters

It is no discredit to the maternal organism to say that this apparent benefit to her body was brought about through the stimulus of the fetus itself. This has been proved for the mammary glands by Herrmann [65] and others, the hormone coming from the placenta, and it seems reasonable to hope that we shall some day find a stimulus to growth and development for underdeveloped women by employment of such extracts made from lying-in material.

The story for nitrogen retention may be repeated with some variations for other chemical elements, phosphorus, sulphur, calcium, magnesium. Hoffström's curves show a substantial gain for each element except calcium. This should be borne in mind in relation to a possible acidosis to be mentioned later.

Hoffström's beautiful work, which required two years for its completion after the materials were collected, together with Wilson's work, may be truly said to demonstrate that, so far from being a sacrifice of the individual for the good of the species, gestation normally may be looked upon as a means employed by the species for the good of the individual (mother).*

Further evidence of physiological adaptation may be found in a study of the *qualitative effects of pregnancy on protein metabolism.* Pregnancy is one of the few conditions studied (fasting is another) in which the distribution of nitrogenous and

* It would be interesting and important to know whether at the end of lactation any part of this acquisition on the part of the mother has been retained.

sulphur compounds in the urine shows any departure from the usual distribution in normal adults on ordinary mixed diets. All of these changes, however, can be explained on physiological grounds. The following changes in the absolute sense have been demonstrated, all referrring to late pregnancy:

1. Urea nitrogen is distinctly lower (Massin,[66] Whitney and Clapp,[67] Mathews,[68] Edgar,[69] Murlin,[70] Murlin and Bailey [71]).

2. Ammonia nitrogen is very slightly, if any, higher

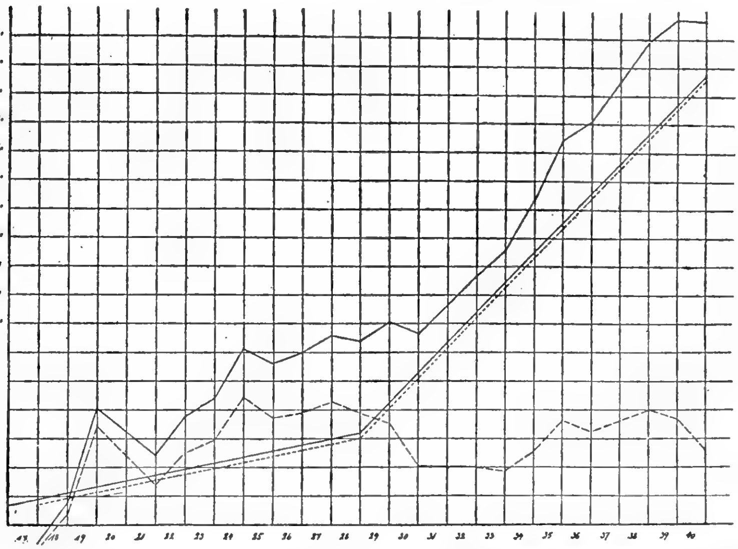


FIG. 4.—Chart showing retention of calcium by Hoffström's case from the seventeenth to the fortieth week of pregnancy. ———, total calcium retained; - - - - -, calcium retained by the fetus (based on Michel's analysis, table 3); — — —, calcium retained by mother's own body.

(Slemons,[72] Falk and Hessky,[73] Murlin,[74] Murlin and Bailey,[71] Gammeltoft,[57] Hasselbalch and Gammeltoft,[75] Lossee and Van Slyke,[63] Wilson [59]).

3. (*a*) Creatinin nitrogen may rise just before parturition (Murlin for dog and woman; not observed three to six weeks antepartum by Van Hoogenhuyse and ten Doeschate [76] or Murlin and Bailey); (*b*) creatinin may fall just before parturition (Gammeltoft for rabbit).

4. Creatin appears in urine even on creatin-free diet shortly before parturition (Heynemann,[77] Murlin,[70] Krause,[78] Van Hoogenhuyse and ten Doeschate, Murlin and Bailey, Gammeltoft).

5. Amino-acid nitrogen, as determined by the Van Slyke method, not increased (Lossee and Van Slyke); as determined by Henriques and Sörensen titration method, slightly increased (Falk and Hessky, Murlin and Bailey, Gammeltoft, Wilson).

6. Total purin nitrogen somewhat increased (Murlin and Bailey).

7. Undetermined nitrogen, including polypeptid nitrogen, slightly increased (Ewing and Wolf, Murlin, Murlin and Bailey, Falk and Hessky, Lossee and Van Slyke).

8. Inorganic sulphate sulphur, distinctly lower, and neutral sulphur, only relatively higher (Murlin for dog and woman; Hoffström for woman).

The explanation of the lower urea nitrogen and inorganic sulphate sulphur is found in the retention of materials which, in the absence of the fetus, would be excreted in these forms. The relatively higher creatinin and unoxidized or neutral sulphur, products considered as strictly endogenous, are explained by the lower percentage of the exogenous urea and inorganic sulphate sulphur. The presence of creatin is probably due either to the slight acidosis to be mentioned presently or to the demands of the fetus late in pregnancy for carbohydrate, or both. The slightly higher amino-acid and polypeptid nitrogen and undetermined nitrogen may not be strictly reciprocal with the urea nitrogen, for they are lowered in the absolute sense immediately after parturition. Slight increase of these substances does not signify deficient deamination by the liver, for if the liver were injured, one could scarcely imagine that it would recover immediately after parturition. The sudden drop much more cogently suggests a fetal origin for such bodies. In so far as protein materials must be worked over by the placenta to harmonize them to the purposes of the fetus, there must be rejected materials and these added to the general circulation would in part pass to the kidney before being deaminated, whereas such bodies originating in the alimentary tract have first to pass the liver.

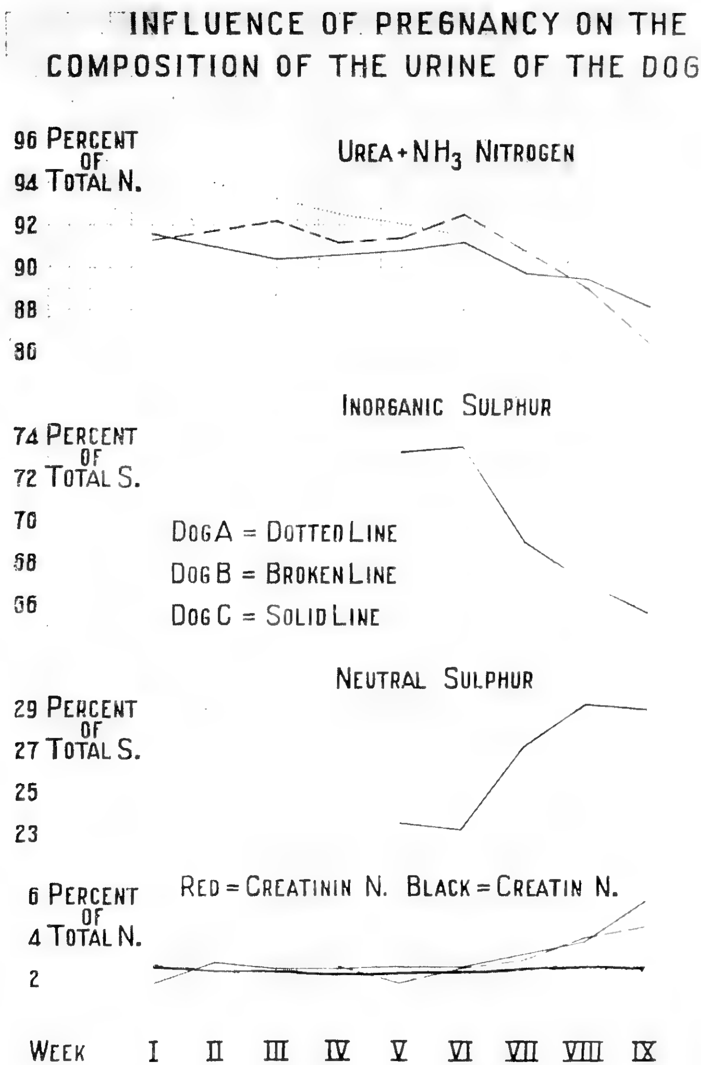


FIG. 5.—Chart showing influence of pregnancy on the *percentage* of urea and ammonia nitrogen, creatin and creatinin nitrogen, and the inorganic and neutral sulphur fractions in the urine of the dog. Compare Figs. 6 and 7. The changes are due to retention of nitrogen which would be excreted as urea in the non-pregnant condition and of sulphur which would be excreted as inorganic or oxidized sulphur.

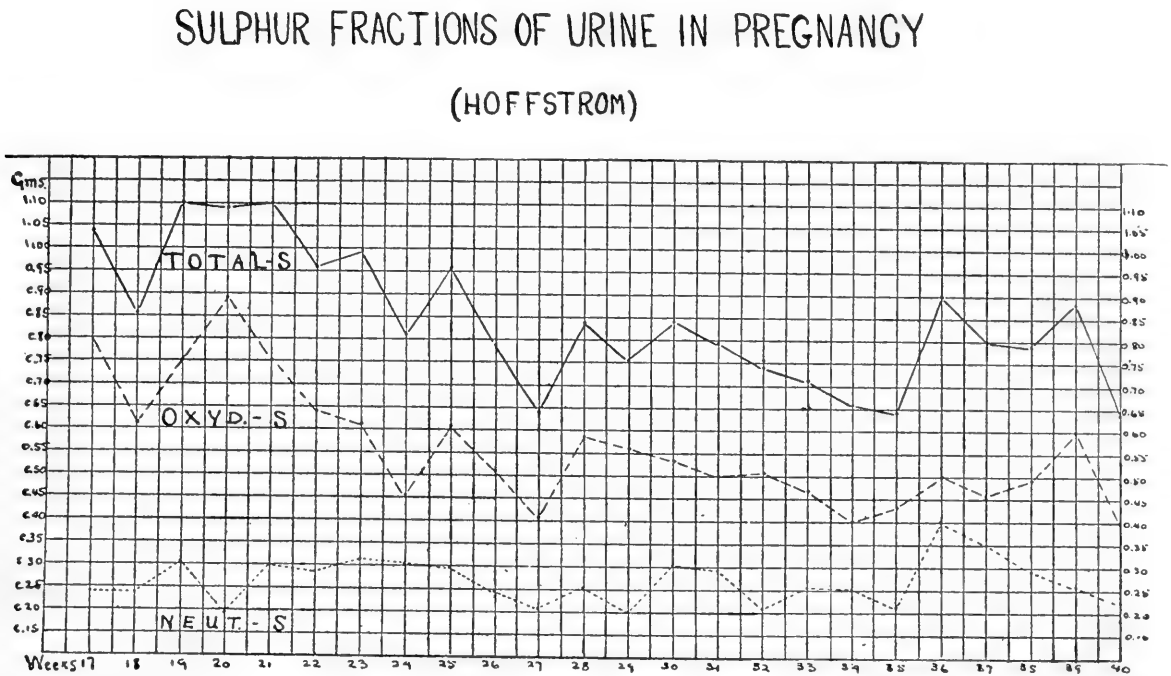
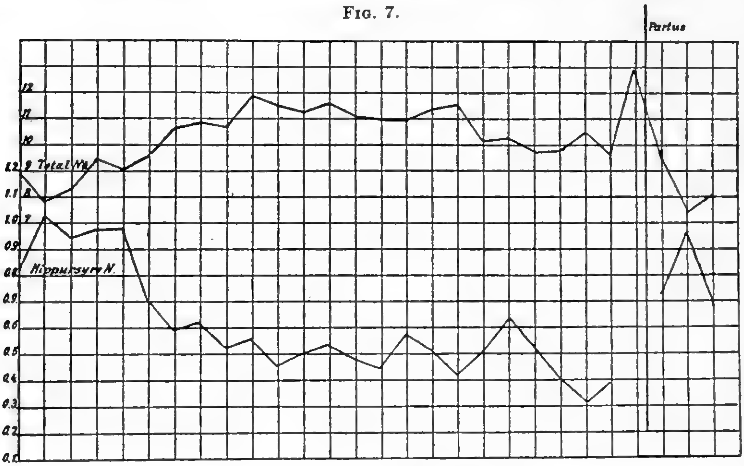


FIG. 6.—Chart showing the distribution of the sulphur fractions in the urine of Hoffström's case from the seventeenth to the fortieth week of pregnancy. The neutral sulphur remains at about the same level (in the absolute sense) throughout. The inorganic sulphate sulphur falls, showing that the sulphur which in the non-pregnant condition would be oxidized is held back by the fetus.

FIG. 7.



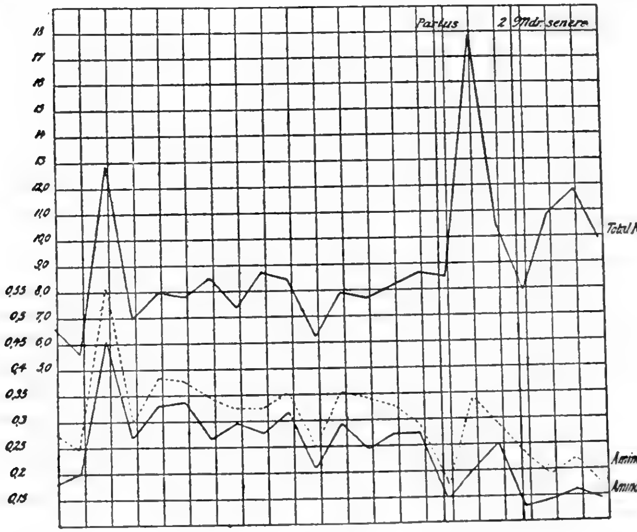


FIG. 8.

FIG. 7.—Chart showing the relation of the hippuric acid nitrogen to the total nitrogen in the urine of a goat during pregnancy (Gammeltoft). The hippuric acid in herbivorous urine corresponds to the urea nitrogen in the urine of other animals. This chart should be compared with Fig. 5, showing the behavior of urea and ammonia nitrogen in the dog.

FIG. 8.—Chart showing relation of mon-amino-acid nitrogen and of total peptid-bound nitrogen to total nitrogen in the urine for last two months of a normal pregnancy (Gammeltoft). Note the fall in amino bodies immediately after parturition simultaneously with the great rise in total nitrogen.

The ammonia nitrogen deserves more than passing mention. Ten years ago this fraction of the nitrogen in the urine suddenly assumed large proportions in obstetrical literature because of its supposed value as an index of hepatic inefficiency. This estimate has not been maintained because, on the one hand, known pathological lesions of the liver have not been shown to produce high ammonia and, on the other, because the researches of Underhill and Rand,[79] Heynemann, Murlin and Bailey, and Lossee and Van Slyke, have made it very doubtful whether the high ammonia of pernicious vomiting is ever to be ascribed to anything more than starvation and depletion by the fetus; they have shown further that eclampsia may be accompanied by no increase in ammonia and that large errors may easily arise from faulty methods of collection and preservation of the urines. The significance of ammonia in the urine is, therefore, restored to its old status; namely, an index of the depletion of fixed bases. Is there such a depletion in normal pregnancy, either by over production of acids and excretion of bases in combination with them, or by withdrawal of bases to the fetus? The position of Murlin and of Heynemann that there is in normal pregnancy no absolute increase, but only a relative one due to lower urea nitrogen, has been substantiated by the most recent investigations of Hasselbalch and Gammeltoft, at Copenhagen, by Wilson, at Johns Hopkins, and by Lossee and Van Slyke, here in New York. The effect of the nitrogen retention upon the ammonia percentage is well illustrated by the following figures from Landsberg. The diet was similar in all cases. Also the effect

TABLE 4.—AMMONIA NITROGEN IN URINE OF PREGNANT AND NON-PREGNANT WOMEN (Landsberg)

	Total N	NH_3-N	Per cent of Total
Average 10 cases pregnant	12.68	0.786	6.2
Average 6 cases non-pregnant	16.03	0.771	4.8

of a severe catharsis is illustrated in one of the cases reported by Murlin and Bailey.

TABLE 5.—EFFECT OF CATHARSIS (Murlin and Bailey)

	Total N	NH_3-N	Per cent of Total	Cr.-N
Normal pregnancy, ninth month....	5.82	0.57	9.8	0.21
After severe catharsis	3.36	0.58	17.3	0.23

In both instances the total ammonia excretion in grams is the same and the higher percentage is due to lower total nitrogen, caused, on the one hand, by retention, and on the other, by a too severe purging which removed the food as well as the waste from the bowel.

The absence of high ammonia in the absolute sense does not, however, preclude the presence of a slight acidosis. The body has other resources than the formation of ammonia for the neutralization of acid. A slight excess of organic acid in the circulation could be compensated by removal of a little more carbonic acid from the blood, while a slight relative acidosis due to diversion of bases to the fetus would produce the same net effect indirectly by diminishing the carbon dioxid-carrying power of the blood. As a matter of fact, Leimdorfer, Novak and Porges [80] and Hasselbalch and Gammeltoft find a lower alveolar tension of carbon dioxid in pregnant women and state that it appears as early as the first month following conception. Lossee and Van Slyke confirm this for late pregnancy by Van Slyke's method for the carbon dioxide-combining power. The delicate regulation of the actual hydrogen-ion concentration without drawing upon the ammonia mechanism is beautifully illustrated in Hasselbalch and Gammeltoft's work. They report simultaneous determinations upon urine, blood and alveolar air, both before and after parturition, for eleven cases of normal pregnancy.

TABLE 6.—ACIDOSIS OF NORMAL PREGNANCY. AVERAGE OF ELEVEN CASES (Hasselbalch and Gammeltoft)

	Ante Partum	Post Partum
Urine:		
Total Nitrogen ....(grams)	9.2	11.4
NH_3–N ....(grams)	0.55	0.57
Per cent of Total	5.9	4.9
Blood:		
P_H at 30 mm. Tension......................	7.44	7.49
Corrected for Actual Tension	7.44	7.44
Alveolar Air:		
CO_2 Tension (mm.Hg.)	31.3	39.5

The ammonia before parturition is not higher than after but there is at any given tension of carbon dioxid a higher concentration of hydrogen (lower P_H). Corrected for the observed carbon dioxid-tension in the blood drawn, the concentration of hydrogen is exactly equalized. The slight decrease in carbon dioxid-carrying power of the blood (for any given tension of that gas), as compared with the value which is restored after parturition, is exactly compensated by the greater activity of the respiratory mechanism for removal of the carbon dioxid. Hasselbalch, indeed, has shown that the respiratory regulation of the hydrogen-ion concentration is brought about by an increased sensitivity of the respiratory center to the presence of carbon dioxid. The regulation, therefore, is perfectly automatic.

METABOLISM IN THE PUERPERIUM

The first few months of pregnancy may well be described as a contest between the new organism and the old. The new, with its intensely active proteolytic enzymes, is concerned with getting a foothold upon a supply of nourishment. When its activities become too aggressive or the maternal reaction is not quite up to normal, the result may be a severe disharmony. Otherwise, the contest results in a compromise, the mother conferring nutriment and protection in exchange for stimulus to her own organs. But there comes, by what precise causes we shall not stop now to inquire, a limit to this tolerance on the part of the mother, and parturition ensues. What readjustments in the metabolism of the mother do we witness after the separation? The necessity for retention of building materials is, for the immediate future, somewhat less even though the child is to be nourished by the mother; for intrauterine life was dependent upon the maintenance of many adventitious structures, some of which, being no longer required, are thrown away, and others are restored to pregestation size. The first change we might expect, therefore, would be the disappearance of the positive balance for the several elements, which has been described, and its replacement by a negative balance. Only the nitrogen balance has been studied in women during the involution period but it is safe to say that

what holds for this element will in general be true of the others; sulphur, phosphorus, calcium, magnesium.

The chief source at least for the extra nitrogen found in the urine is the uterus itself; for Slemons [81] has shown that a woman who withstood Caesarean section and prolonged anesthesia excreted only 40 grams more nitrogen in the urine during the puerperal period of twenty days than during the post-puerperium, while a uterus removed from another woman of the same race contained 38 grams. Making some allowance for the effects of anesthesia, some for the difference between the two uteri, and some for lochia, the correspondence is as close as could be expected. The woman whose uterus was removed by the Porro operation should have excreted about 35 grams less than the one whose uterus went through involution following conservative Caesarean section. Less than this difference would have indicated the loss of nitrogen from other tissues. As a matter of fact, while on a similar diet and after anesthesia of similar duration, she excreted 61 grams less. The discrepancy, Slemons thinks, was attributed to other extraneous differences between the two cases. The observation proves at least that nitrogen is not lost from other organs. In view of the enormous total retention of nitrogen outside of the genital tract which has been made out by Hoffström, Landsberg and Wilson, the occasion for surprise is that all of it apparently is conserved during the puerperium, doubtless in the interest of lactation. The nitrogen output reaches its maximum on the fifth day for the dog and on the sixth or seventh for the woman.

Taking the period just before delivery as a standard, the following changes in the distribution of nitrogen in the absolute sense are observed in the period immediately following: The urea nitrogen rises, the creatin rises, the ammonia remains the same, the creatinin falls, and the monamino-acid nitrogen falls. In percentage of the total the urea nitrogen may either rise or fall, depending upon the adequacy of the diet in the first few days after delivery and upon the rate of retention for milk production, as against the rate of involution a little later. Where sufficient care is exercised in the collection of the urine, it will be

found in normal cases that the percentage of ammonia always falls, owing to the rise in the total nitrogen. There is no acidosis characteristic of the puerperium. The absolute decline in the creatinin excretion, Longridge [82] believes, is explained by the reduction in mass of active muscle. The creatin fraction only calls for more extended remarks. Having in mind that creatin had been identified in smooth muscle [83] and that in wasting diseases, muscular dystrophy especially, creatin always is found in the urine, Shaffer [84] and I [85] independently ascribed the creatinuria of the puerperal period to the involution of the uterus, and this view was generally adopted. Mellanby,[86] however, sharply challenged this explanation by showing that a woman may excrete even more creatin after the uterus is removed than another who has been through essentially the same operative ordeal without hysterectomy and the facts have since been corroborated by Morse.[87]

The observations of both Mellanby and Morse leave something to be desired in the way of control of the diet. The *experimentum crucis* is, of course, a very exacting one. Both patients, the one after simple Caesarean section, and the other after the Porro operation, must be upon an adequate diet, which, of course, should be creatin-free, from the moment food can be taken. Most operated patients of this character could not take a diet adequate to protect the body against loss of creatin and if they could the further difficulty would at once be presented as to whether the more radical operation would not of itself cause loss of creatin. Both observers have, in fact, proved too much. In order to show that the creatin does not have its origin in the uterus, it would be sufficient only to prove that just as much creatin is excreted by a patient following removal of the organ as by another in whom it is left in place. But in all three cases, one studied by Mellanby and two by Morse, *more creatin was found after the radical operation*, a fact which points to less complete nutrition or some other vitiating circumstance of the radical operation. Once this is admitted, does not the validity of the whole comparison fall down?

Soon after Mellanby's paper appeared, Bailey and I at-

tempted to test the matter on dogs, by removing the uterus several days after parturition, maintaining the animal both before and after operation on the same creatin-free diet. Out of four attempts only one succeeded; *i.e.,* in only one case did we succeed in inducing the animal to consume an adequate diet of creatin-free materials throughout. This dog showed the higher excretion of creatin after the operation, but we are almost convinced that the result was due to the acidosis incident to anesthesia.

Mellanby offers some evidence that the excretion of creatin is in some way associated with the activity of the mammary glands; for example, when the milk is delayed, creatin in the urine does not appear and the curve of creatin excretion runs parallel with the curve of milk production.

III. THE ENERGY METABOLISM

Growth and maintenance of cells are dependent upon two fundamental properties of protoplasm which ordinarily are regarded as quite distinct because in the adult they may vary quite independently. In the developing ovum, however, we are beginning to see how they may be very closely related. One of these properties, the ability to attract and incorporate into its own structure and thus vitalize germane materials, has been discussed for the fetus and the effects of this intrauterine growth upon the protein metabolism of the mother have now been passed in review. The other fundamental property is the ability to activate oxygen so that without raising the temperature to the kindling point for the oxidizable materials, energy may be set free by oxidation.

Growth is even more dependent upon oxidation than is mere maintenance of the body. Warburg[88] has made the interesting suggestion that the purpose served by oxidation in cells which do no external work, is to maintain the internal structure of the cell. Certain properties of semi-permeable membranes, such as the electric charge, are preserved, thereby preventing mixing of the constituents by diffusion. Internal structure, Warburg believes, is necessary also to provide surfaces for condensation of the catalysts which are active in the cell processes. Now Meyer-

hof[89] has shown that the "caloric quotient" of developing eggs is considerably lower than in adult organisms. By "caloric quotient" one means the number of gram calories of heat produced for each milligram of oxygen consumed. In resting adult organisms it varies from 3.2, where protein is the source of energy, to 3.5, where carbohydrate is the source, the value for fat lying between these extremes. In developing sea urchin eggs it is 2.5 to 2.9, thus indicating that oxygen is being used extensively for some other purpose than heat production. Following Warburg's suggestion, we may suppose, then, that more oxygen is needed to maintain the structure because more catalysts are at work or are working more actively, or, as Lyon[90] has suggested, the oxygen may be used directly in the synthetic processes of growth. On either supposition we see how the substance metabolism involving transformation chiefly of proteins may be closely related in the embryo to energy metabolism.

Warburg[91] has recently repeated many of his earlier studies on the respiratory exchange of the sea urchin egg and has confirmed them in all essential respects. The oxygen absorption of the unfertilized ovum is about five hundred times that of the sperm cell of the same species. But when the two cells unite in the act of fertilization the oxygen absorption goes up to about 3500 times that of the sperm. This increase to sevenfold the metabolism of the unfertilized egg takes place in ten minutes. At the end of six hours it is twelve times and at the end of twenty-four hours, when the gastrula stage is reached, it is twenty-five times the absorption before fertilization.

It is safe to say that something like this happens in the mammalian ovum upon fertilization. But we can not study the metabolism of a mammalian ovum at such an early stage. It is not until well beyond the middle of pregnancy that the respiratory exchange of the simple fetus is large enough to be measured by existing means. We shall limit our inquiry here to two questions: (1) what kinds of material are oxidized to furnish the energy in the fetus and newborn; and (2) how much energy is thus set free in the pregnant woman and the new-born child in comparison with the adult?

THE RESPIRATORY QUOTIENT OF DEVELOPMENT

Qualitative differences in the energy metabolism of the embryo depend upon the kind of material supplied by the mother. The hen supplies only fat and protein in the egg; hence the respiratory quotient during development into the chick can never be higher than 0.78. Chemical studies of eggs before and after incubation by Liebermann,[92] the calorimetric determinations of the heat of combustion before and after incubation by Tangl,[93] and the metabolism studies (using both direct and indirect methods) by Bohr and Hasselbalch,[94] all agree in showing that the material oxidized in the development of the chick is fat.

Regarding the source of energy for mammalian development our information is extremely scanty. Cohnstein and Zuntz,[17] analyzing the blood of the umbilical vein and artery of the sheep embryo, found respiratory quotients of 1.0 and 1.6, respectively, in two cases. Bohr,[95] measuring the total respiratory exchange of the pregnant guinea-pig before and after clamping an umbilical cord, noted differences which gave a respiratory quotient for the embryo in the neighborhood of unity. These are all the recorded observations on the respiratory exchange of the fetus directly. Such as they are, they indicate plainly that the source of the energy is carbohydrate, the most readily diffusible of all the food-stuffs. Several observers have noted a rising respiratory quotient during pregnancy in both lower animals and the human subject and there is no doubt, from the observations of Carpenter and Murlin [96] and Hasselbalch,[97] that the quotient is higher just before parturition than just after, but it is not certain to what extent the limited diet usually allowed the puerperal mother in the first days after delivery is responsible for the difference.

The respiratory quotient of the new-born has been for some twenty-five years a matter of recurrent interest. The earliest observations by Mensi, Scherer and Babak have turned out to be wholly untrustworthy because of imperfect technique. Murlin [98] reported in 1908 that the respiratory quotient of the new-born puppy was in the neighborhood of unity. Hasselbalch,[99] in 1904, and Weis,[100] in 1908, were the first to observe that the quotient

for the new-born infant, also, is high, often in the neighborhood of unity, and indicating the combustion of carbohydrate. Without knowing of these results because they were published in obscure places, Baily and Murlin [101] obtained quotients of the same character in two infants observed at six hours of age. They confirmed Hasselbalch's observation, also, that the quotient falls rapidly after the first few hours, and on the second day before food was given they found it slightly below the quotient of pure fat combustion and indicating a certain degree of starvation acidosis. The interpretation placed upon these observations by Hasselbalch and by Bailey and Murlin was essentially the same; namely, that the child is born with a sufficient reserve of carbohydrate to supply its energy requirement for a portion of the first day, but that this supply is quickly exhausted and the child should be fed very early. Benedict and Talbot,[102] in a long series of determinations on new-born infants, however, have failed to find the quotients uniformly high in the first hours, although they admit that the majority of the cases observed within eight hours of birth gave quotients above 0.80, whereas the majority of those observed after eight hours gave quotients below 0.80. Two reasons for the discrepancy found by the different authors may be mentioned. One of these is given by Hasselbalch; namely, the state of nutrition and the maturity of the child when born. In his series Hasselbalch was certain that the better the nutritive condition of the infant, the higher was the quotient, and the average respiratory quotient for prematurely born infants was below that of infants born at term. The other reason, I believe, is found in the level of the blood sugar at the time the child is born. When the mother has a severe labor or when an anesthetic is necessary, the blood sugar of the mother, as well as that of the fetus, according to Morriss,[18] rises. In the former circumstance it may rise in the fetal blood to 0.12 per cent. and in the latter to as much as 0.14 per cent. These are distinct degrees of hyperglycemia and might very well sustain the respiratory quotient at an unusual level for several hours. Hence, we might well expect the quotient of the new-born, following a prolonged and severe labor, to be high. Indeed, Hasselbalch draws

especial attention to one of his prematurely born infants delivered by a forceps operation, because it gave a quotient higher than others of like age. When we remember that large, well-developed babies cause more prolonged labor than small or prematurely born babies, we find another reason for Hasselbalch's discovery that the former present the higher quotients.

QUANTITY OF ENERGY REQUIRED IN DEVELOPMENT

With the exception of a few wholly untrustworthy observations of Cohnstein and Zuntz [17] on the embryo sheep, in which the authors undertook to measure the total oxidation by analysis of blood drawn from the umbilical vein and artery, the only direct studies of the energy metabolism of the mammalian offspring before birth is that of Bohr.[95] Bohr operated a pregnant guinea-pig so as to expose the umbilical vessels. With the anesthetized mother immersed in a warm bath of salt solution he then measured the respiratory exchange through the maternal system before and after clamping one of the umbilical cords so as to exclude entirely the one fetus. From the differences obtained he calculated the metabolism of the young near term at 509 cc. of carbon dioxid given off per kilogram an hour as against 462 cc. for the mother, an increase of about ten per cent.

Rubner [103] in 1908 expressed the belief that his law of surface area applied to the embryo as well as to the new-born. Assuming the average weight of each individual at birth to be eight per cent of that of the mother, he calculated that the energy metabolism per unit of weight of any new-born mammal would be approximately twice that of the mother. Because the fetus is much less active than the new-born, its metabolism, so Rubner held, should be considerably less than this, which indeed Bohr's fragmentary results indicate is the case.

We shall return to the new-born later. Meantime one gets very little help either from Rubner or Bohr in forecasting what the effect of the fetus would be on the total metabolism of the mother. Granting that its energy requirement is greater than the same weight of maternal tissue, we must remember that a large part of the increased weight at the culmination of preg-

TABLE SHOWING THAT EXTRA HEAT PRODUCTION OF PREGNANCY IS PROPORTIONAL TO WEIGHT OF OFFSPRING TO BE BORN

DAY FROM PARTURITION	WEIGHT IN KG.	TEMPERATURE OF CAGE	CALORIES IN FOOD	TOTAL ENERGY PRODUCED	ENERGY RETAINED
FIRST PREGNANCY					
THIRD BEFORE (JUNE 23)	14.5	28.0°C	907.4	551.3	356.1
PARTURITION (JUNE 26)	ONE PUPPY BORN: WEIGHT, 280 GM.				
SEXUAL REST; AFTER LACTATION					
THREE WEEKS AFTER PARTURITION (JULY 15)	13.78	28.1°C	907.4	505.3	402.1
SECOND PREGNANCY					
THIRD BEFORE (DEC. 11)	16.86	27.1°C	907.4	763.8	143.6
PARTURITION (DEC. 14)	FIVE PUPPIES BORN: WEIGHT, 1560 GM.				

551.3 − 505.3 = 46.0 CALORIES FOR 1 PUPPY WEIGHING 280 GM.

763.8 − 505.3 = 258.5 CALORIES FOR 5 PUPPIES WEIGHING 1560 GM.

$$\frac{280}{46} = \frac{1560}{258.5}$$

FIG. 9.—Extra heat production in a dog just before parturition in two different pregnancies. From the first, one pup was born; from the second, five.

nancy takes little or no part in the metabolism; fluids, none at all; membranes and cord, next to none; placenta, uterus and mammaries, probably not more than so much maternal matter. The net effect, therefore, would be a sort of algebraic sum of high, low and medium metabolism added to that of the mother's. As a matter of fact, the earlier observations on pregnant animals give conflicting results. While Reprew,[51] working with rabbits, guinea-pigs and a dog, reported no increase in metabolism per unit of weight, Oddi and Vicarelli,[104] working with mice, found a marked increase. Magnus-Levy [105] also, in the first observations on the energy metabolism of the pregnant woman ever recorded, noted an increase in oxygen absorption from 2.8 cc. per kilogram a minute in the third month to 3.3 cc. in the ninth, a rise of 17 per cent.

My own observations [106] on the dog made on the third day before parturition on a pregnancy from which only one pup was born, show an increase of six per cent per unit of weight over that of complete sexual rest, while on the corresponding day of a later pregnancy in the same dog from which five pups were born the increase was 28 per cent. The extra metabolism was proportional to the weight of the new-born delivered.

In the woman pregnant with a single fetus the observations of Zuntz,[107] Carpenter and Murlin and Hasselbalch agree in showing an extra metabolism near term of about four per cent over that of the same woman or other women in complete sexual rest. All of these authors surmise that this is scarcely more than may be accounted for by the increased respiratory activity necessary to preserve the hydrogen-ion concentration of the mother's blood. It is evident, then, that the total product of conception added to the mother's body functions as so much maternal tissue—the higher metabolism of the embryo being just counterbalanced by the inactive and relatively inactive structures.

From observations on the dog above referred to, the extra metabolism on the third day previous to parturition was equivalent to 164 calories in the first pregnancy and 165 calories in the second per kilogram of pup delivered three days later. Assuming the applicability of Rubner's law of surfaces to the

new-born pup, with the same constants as for the adult dog, it was calculated that the theoretical metabolism necessary to maintain in muscular rest a new-born pup weighing what the fetus actually weighed would be just equal to the extra metabolism of pregnancy. In other words, if the new-born pup were to lie perfectly still and sleep as quietly as the fetus does, the increased metabolism at room temperature over the metabolism at its mother's body temperature would just compensate the metabolism of the placenta, uterine wall, etc., and the total requisition placed upon the mother for maintenance of the new-born by food from her mammary glands would not, for the first days at least, exceed the requisition made upon her body in the last days of pregnancy by way of the placenta. Here appeared a very important principle of adaptation, the requirements of the new-born being just equal to the requirements of the total product of conception, accessory structures included, just before parturition. It was impossible to demonstrate the principle with absolute certainty on the dog because of the mother dog's anxiety for the offspring in the first days after parturition. It was demonstrated a year later, however, by Carpenter and Murlin [96] on three cases of human pregnancy at the nutrition laboratory in Boston. These three cases, two primiparæ and one multipara, were observed in the bed calorimeter for some three weeks previous to parturition and mother and child together were placed in the calorimeter again as soon as possible thereafter. The mothers were soon trained to lie perfectly still and by keeping the infants awake for several hours just before the calorimeter periods, they were readily induced to sleep throughout or nearly throughout the observational period of two to three hours. In two of the cases the comparison of antepartum and postpartum metabolism of mother and offspring together showed a difference of less than one per cent. The other case showed an increase of seven per cent, partly because the antepartum observations did not occur closer than the thirteenth day before delivery and partly because the child cried on two out of three occasions while in the calorimeter in the later experiments. Ruling out the factor of muscular activity as we are able to do in the two

TABLE 7.—ENERGY METABOLISM OF MOTHER AND CHILD TOGETHER BEFORE AND AFTER PARTURITION (Carpenter and Murlin)

Case.	Respiratory Exchange.				Energy Production.				Calories per hour.	
Mean of all days before and after delivery.	Average body. temp. °C.	CO_2 gm. per hour.	O_2 gm. per hour.	R. Q.	a—Direct.	b—Indirect.	$\frac{a+b}{2}$	% difference.	per kg.	% difference.
Case 1—										
1st, 4th and 6th before delivery	36.75	21.3	18.4	.85	60.0	61.3	60.7	.....	0.96	......
2d, 5th, 12th, 14th and 17th after delivery	36.9	20.2	18.5	.80	61.2	61.2	61.2	+.87	1.11	+15.6
Case 2—										
13th, 17th, 19th, 20th and 22d before delivery	36.68	22.3	19.6	.83	63.6	65.9	64.7	.....	1.11	......
2d, 5th and 11th after delivery	36.8	21.7	20.4	.78	71.1	67.5	69.3	+7.1	1.32	+18.9
Case 3—										
1st, 3d, 17th, 21st and 24th before delivery	36.64	23.9	20.2	.86	72.2	68.7	70.6	.....	1.02	......
4th, 8th and 11th after delivery	37.23	23.1	20.3	.81	70.8	68.6	69.7	— .9	1.11	+ 8.8

cases, the curve of total energy metabolism of the mother and offspring suffered no deflection at parturition. It is a remarkable fact that the increase in oxidation in the child's body when it passes from the warm environment of its mother's uterus to the colder environment of the outside world (in bed beside its mother), supplying its oxygen now by its own lungs instead of from the mother's placenta, should so nearly compensate the oxidation in the accessory structures which supported it *in utero*. Just how much the child's metabolism is altered by the changed environment and changed circulation we have no certain means of knowing. That it is considerable, that the change represents, indeed, a turning-point, in the quantitative sense as well as in the mode, of nutrition, is evident from what has been said already as well as from what will follow immediately. The demands upon the mother's digestive system, however, are not greater. She is called upon to supply the same amount of energy in potential form to herself and child immediately after parturition that she did to herself and child immediately before.

The rate of oxidation or heat production per unit of weight for the puerperal woman in these three cases was eleven per cent higher than the average for eight non-pregnant women and seven per cent. higher than that of the same subjects just before delivery, a difference which may be ascribed in part to the increased activity of the mammary glands and in part to the stimulating effect of the products of involution. Since these products are protein in nature they would unquestionably stimulate metabolism in the same way as Lusk [108] has shown for the amino-acids.

METABOLISM OF THE NEW-BORN

The energy production of a grown person in health and while resting in bed may be stated as approximately 1.0 calory per kilo of body weight per hour (Du Bois). The average for eight normal non-pregnant women between the ages of eighteen and fifty-five years under these conditions was found by Carpenter and Murlin to be 0.99 calory per kilo an hour and the average for three normal puerperal women was 1.09 calories.

The only comparison ever made between the metabolism of

the new-born infant and its puerperal mother was reported by Carpenter and Murlin in the work to which reference has been made. The infant's metabolism was measured by difference between the metabolism of mother and child taken together and that of the mother taken alone. The average age of the infants at the time of the observations on the mother alone was ten days. The metabolism was found to be 2.8 calories per kilo an hour or 2.5 times that of the mother. When we compare the metabolism per unit of body surface, as calculated by Meeh's formula, we find that of the child somewhat less than that of the mother. Nothing could better illustrate the applicability of Rubner's law of surface to persons of different size and widely different physiological conditions than the data from this comparison. The pregnant woman just before delivery, the same woman two weeks after delivery, weighing 9 to 10 kilograms less, and the child weighing one-sixteenth to one-twentieth the weight of the mother —all produce the same amount of heat per unit of surface.

Table 8.—Metabolism Before and After Parturition. The Metabolism of the Child Was Determined by Difference (Carpenter and Murlin)

Case I:	Weight in kg.	Calories per Hour.	Calories per Sq. M. (Meeh).	Calories. per kg. per hour
Before parturition	63.0	60.7	31.4	0.96
After parturition	51.4	53.9	31.7	1.05
Difference	11.6	6.8		
Child	2.7	7.3	30.5	2.70
Case II:				
Before parturition	58.0	64.7	35.1	1.11
After parturition	48.5	59.0	36.2	1.21
Difference	9.5	5.4		
Child	3.4	9.8	34.9	2.88
Case III:				
Before parturition	69.1	70.6	34.0	1.02
After parturition	60.1	60.4	31.9	1.00
Difference	9.0	10.2		
Child	3.2	9.3	34.8	2.90
Average:				
Before parturition	63.0	65.1	33.5	1.03
After parturition	53.0	58.1	33.3	1.09

As sometimes happens in scientific work, the beauty of a comparison of this sort is marred slightly by more accurate data. Since this work was done, nearly eight years ago, observations by Benedict and Talbot and by Bailey and Murlin have shown that the metabolism of the sleeping, new-born infant is nearer two calories than 2.8 per kilogram and hour and 25 calories rather than 30 per square meter and hour, in both respects distinctly lower than the metabolism under similar conditions for the adult. Note that this fulfills exactly the estimate made by Rubner on purely *a priori* ground.

Bailey and Murlin were fortunate enough to have as subjects two infants of widely different body weight born on the same day just three hours apart, so that it was possible to study them successively at exactly the same age. This comparison illustrates the influence of body fat on the heat production. The larger infant has the lower metabolism on the basis of weight, but the two have nearly the same metabolism on the basis of surface.

TABLE 9.—ENERGY METABOLISM OF TWO NEW-BORN INFANTS (Bailey and Murlin)

Weight, kgm.	Age hours	R. Q.	Cal. per hour	Cal. per kgm. and hour	Cal. per square meter and hour (Meeh)
W. 2.9	6	1.12	5.649	1.94	23.67
B. 4.6	6	0.85	6.724	1.46	20.43
W. 2.82	31	0.66	6.255	2.22	26.54
B. 4.49	31	0.67	8.704	1.94	26.87
W. 2.75	80	0.70	5.972	2.18	25.57
B. 4.27	80	0.73	7.101	1.66	22.67
W. 2.75	104	0.70	5.252	1.83	21.85
B. 4.27	104	0.73	7.500	1.77	23.47
W. Average	...	....	5.782	2.04	24.43
B. Average	...	....	7.514	1.70	23.36

Benedict and Talbot [109] explained such differences as this on the assumption that fat replaces active tissue. They said, therefore, that the lean infant has a higher metabolism per unit of weight than the fat one because he has relatively more active tissue. It turns out, however, as we were able to show, that fat does not replace active tissue but replaces water. Hence, we are driven back upon the old explanation which Rubner himself gave;

namely, that the lean infant has the higher metabolism because he loses heat faster. He has a larger surface in proportion to weight and, since it is through the surface that heat is lost, it will be in proportion to surface that heat must be produced if the body temperature is to remain constant.

A comparison of the energy metabolism of infants through the first year of postnatal life made by Benedict and Talbot and by Murlin and Hoobler [110] reveals a rapidly progressing increase. Starting at a level below that of the adult, the nursling reaches the adult level at about the second month, and from this time on, while traversing the period of most rapid growth, the period of highest milk consumption, it arrives at the apex of the metabolism curve, somewhere between one and two years. From this point on to old age (with the exception of a slight mound at the time of puberty) the rate of oxidation in the resting body is steadily receding.

This lecture opened with emphasis upon the independence of the embryo. The enzymes which enable it to secure materials for its own nourishment from the mother are really a part of the mechanism of heredity. After producing the ovum the mother has no further influence on the hereditary factors. The enzymes of the embryo, however, can act only on certain proteins—the proteins of its own species.

After a period which may well be called *parasitism* the new and the old organisms become accommodated one to the other and enjoy a period of what Bar has denominated "harmonious symbiosis." The harmony applies to both substance and energy metabolism. The maternal metabolism is nicely adapted to the physiological alteration due to pregnancy and the fetus, with all its adnexa, asks for no more in the way of energy than does the same weight of maternal tissue. We conclude now by calling attention once more to signs of an independent behavior in the metabolism of the offspring. The low rate immediately after birth is probably due to the fact that the heat regulating mechanism is not yet complete; the higher rate beyond the second or third month is doubtless related to the more active growth.

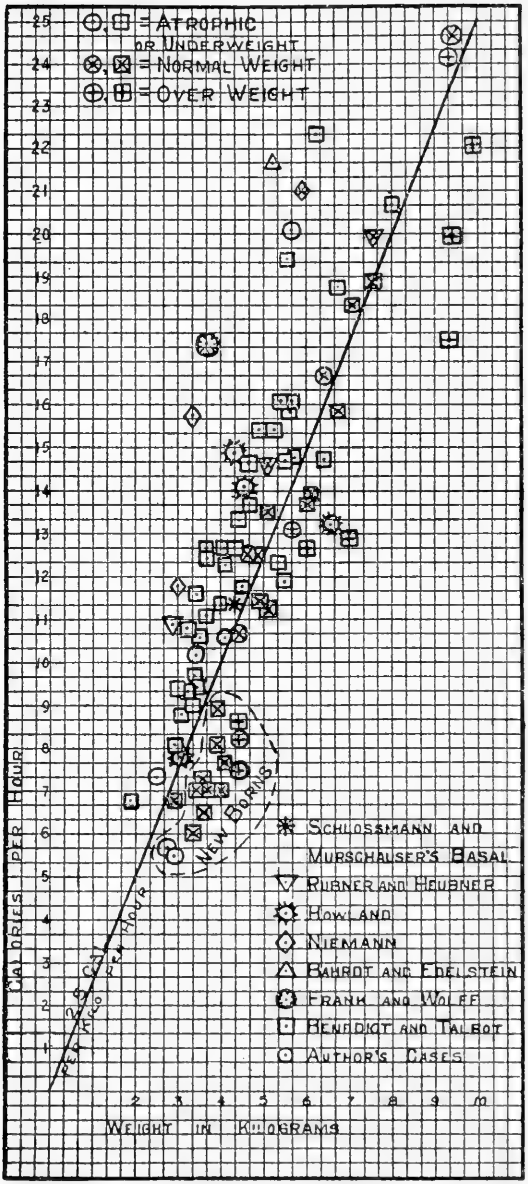


Fig. 10.—Chart from Murlin and Hoobler, showing relation of heat production to body weight in infants. Methods of von Pettenkofer or Regnault-Reiset.

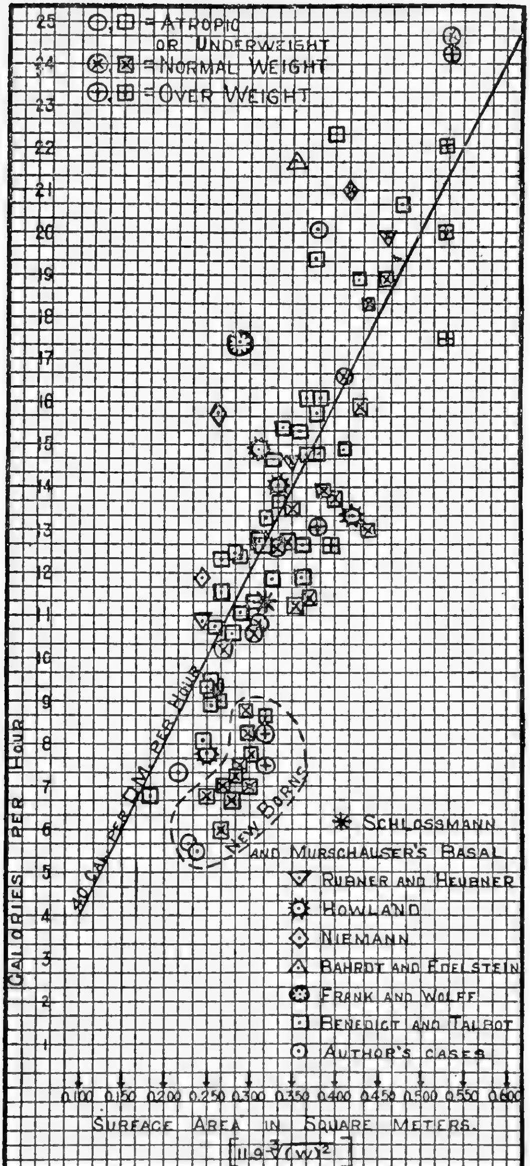


FIG. 11.—Chart from Murlin and Hoobler showing relation of heat production to skin surface in infants. Methods of von Pettenkofer or Regnault-Reiset.

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CARDIAC DYSPNEA*

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EACH of the milestones reached in the continually advancing progress of clinical medicine corresponds closely to some forward step taken in what have come to be known as the "fundamental sciences." A new technical method or a new point of view which opens a fresh way of approach in anatomy, physiology, chemistry or biology is quickly seized upon by the physician in the hope that it may prove to be an addition to his armamentarium which will aid him to gain new knowledge of disease—its mechanism—its recognition—and its cure. The era inaugurated by Virchow gave to us as accurate a conception of the pathological morphology of the commoner diseases as the methods thus far developed would allow, and the last decade in clinical medicine has belonged essentially to biology and physiology. The study of dead form has largely given way to the study of living processes, —the growth of microorganisms and the abnormalities of function produced in cells and organs under various conditions of disease. The significant rôle played by physiology is manifest in many fields of clinical medicine, and the application of the methods and instruments of the physiological laboratory to the study of patients in the wards has broadened and in some instances revolutionized our conception of human pathology.

In scarcely any field has this affiliation between physiology and clinical medicine produced more interesting and stimulating results than in the study of the respiration. The application of modern methods permits the accurate determination of oxygen consumption, carbon dioxid production, the respiratory quotient, and heat production. With their aid we are rapidly gaining

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insight into the more fundamental changes of the intermediary metabolism which are met with in disease. The adaptation of recent physiological researches on the chemical control of the respiratory centre has led directly to the use of methods for determining the carbon dioxid content of the alveolar air in the study of disease. From this, and from analogous methods, we have learned much about those pathological conditions in which acidosis is a significant feature. Neither in the study of the gaseous exchange nor of the alveolar air, however, is the interest focused primarily on the respiration itself. Just as the urine is of value in the investigation of nitrogenous metabolism because of the end products which it contains, so the expired air and the alveolar air are chiefly of interest because they serve as indices of the intermediary metabolism. The respiration itself, the various forms which may occur in disease, the factors which may influence it and limit its efficiency,—these have occupied comparatively little attention. It is to some of these changes, and more especially to a consideration of the causes of the dyspnea which occurs in association with heart disease that the present paper is directed.

Before proceeding to a discussion of those factors which enter into the production of dyspnea, it will be well to state briefly the exact significance of the term itself. As ordinarily used the word is applied loosely to various abnormal types of respiration. Thus not infrequently rapid breathing or tacchypnea is referred to as dyspnea, while even more often the increase of rate and depth of respiration which constitutes hyperpnea is characterized as dyspnea. Neither condition is, however, necessarily synonymous with dyspnea. Dyspnea, as the derivation of the word indicates, is a difficult or labored breathing, and there is implied in it an element of subjective discomfort. Hyperpnea on the other hand, merely signifies an increase above the normal value for the subject at rest in the volume of air breathed. Such an increase of the pulmonary ventilation, or as it is commonly called, of the minute-volume of air breathed may be due to a more rapid respiration or to a deepening of the respiration, but usually both factors take part in it. Whether or not in any

given instance the hyperpnea will amount to a true dyspnea depends on the degree to which the pulmonary ventilation is increased, and on the ability of the subject to raise his minute-volume to that degree easily. As will be seen, anything which prevents a person from increasing his pulmonary ventilation in a normal manner, will be an element in increasing his tendency to dyspnea.

It is extremely difficult to analyze with accuracy the fundamental cause of the subjective sensation which we know as dyspnea. How much is it due to fatigue of the muscles of respiration? How much is it due to a functional insufficiency of the respiration resulting in an inadequate oxygen supply to the tissues, and an incomplete removal of the waste products of metabolism? Without doubt both factors are involved and one is confronted by a vicious circle, in which waste products accumulated in the cells and blood augment the stimulus to the respiratory centre, and this in turn makes still greater demands on the already tired muscles of respiration.

In a general consideration of the respiration it is customary to subdivide the subject into two broad phases,—the external respiration, and the internal respiration. The former depends largely on the lungs, and the essential feature of it is that the pulmonary ventilation shall be such as to supply oxygen to the blood in the amounts required by the metabolism of the body, and to provide for the proper removal of the waste carbon dioxid. The internal respiration in which the circulation plays a prominent rôle, is concerned with the exchange of gases between the blood and the cells of the body. It is clear that if either the external or the internal respiration is inadequate to the task imposed upon it, dyspnea may result. Even when the external respiration produces a blood which is wholly normal as it leaves the lungs, there may be an improper gaseous exchange between the blood and the tissues owing to an imperfect internal respiration. Of the internal respiration, which is possibly the more fundamental phase of the respiration, physiologists and chemists know but little, and of its pathology clinicians know, if anything, somewhat less. The methods for studying even so gross a feature

as the rate of the blood flow are still imperfect, and chemical analyses are limited to blood from the peripheral vessels. The whole field of the internal respiration must, therefore, for the present, be left open, and we shall be restricted to a discussion of the conditions affecting the external pulmonary respiration.

One of the chief factors which have aroused interest in the study of the respiration in disease has been the recent advance made in our knowledge concerning the normal control and regulation of the respiration. The old discussion among physiologists as to the nature of the stimulus to the respiration was in a large degree settled by the classical paper of Haldane and Priestley [1] which showed that carbon dioxid is the essential stimulus, and indicated the extreme sensitiveness of the respiratory centre in that a rise of 0.2 per cent of the carbon dioxid content of the alveolar air caused the ventilation to be doubled. Subsequent investigations have tended to broaden this conception and to Winterstein [2] and Hasselbalch [3] is due the chief credit of demonstrating that the respiratory centre responds not to carbon dioxid alone but to any increase of the acid radicles in the blood.

Since the presence of carbon dioxid and of other acids in the blood depends in general on the chemical processes in the body, it is evident that the basic factor in the regulation of the respiration is the metabolism. The respiratory centre controls the movements of the lungs and regulates them so that the pulmonary ventilation keeps pace with the metabolism. In a normal individual at rest, a minute-volume of approximately 5.0 liters of air suffices to remove the excess of carbon dioxid, and to supply sufficient oxygen for the needs of the body. If, however, the subject walks about the room his metabolism rises, more carbon dioxid is formed, the respiratory centre is more highly stimulated and the pulmonary ventilation is increased. The rise in metabolism associated with the walking may require an increase of the minute-volume of air breathed to three or four times its resting value in order that the needs of the tissues for a proper gaseous exchange may

[1] Haldane and Priestley: Jour. of Physiol., 1905, xxxii, 225.
[2] Winterstein: Arch. f. d. ges. Physiol., 1911, cxxxviii, 167.
[3] Hasselbalch: Biochem. Ztschr., 1913, xlvi, 403.

be met. Such an increase in minute-volume is easily brought about by increasing the rate and depth of breathing and, indeed, a normal person is hardly conscious of any change in his respiration when he is breathing 15.0 liters a minute. With severe exercise the metabolism rises much higher, and in addition to the carbon dioxid formed, Ryffel [4] has shown that lactic acid may be produced. Here, then, is an additional stimulus to the respiratory centre. In an attempt to determine how great a pulmonary ventilation normal persons were capable of, a series of observations have been made in association with Mr. F. C. Hall and Miss B. I. Barker. The experiments consisted in having young men,—doctors and medical students,—ride on a stationary bicycle until they were forced to stop on account of shortness of breath. Some of the subjects were athletes in excellent training while others were accustomed to a sedentary life. The subjects breathed through mouth-pieces, and valves were used to separate the inspired from the expired air. The expired air was passed through a Bohr air meter, and its volume measured for each half minute of the time during which the subject was riding. The rate of the respiration was counted from a continuous pneumographic record. The data obtained over each half minute consisted of the respiratory rate, the total volume of air breathed, and the average volume of each individual respiration. While there was a certain amount of variation among the different individuals in that some tended to greater increase of rate and others to greater increase of the depth of breathing, a number of interesting facts were elicited. Over the last minute and one-half of the ride, thus when dyspnea was most marked, and just before having to stop, the minute-volume of air breathed ranged from 47.6 to 80.0 liters. The larger minute-volumes were, of course, in general found in the larger individuals. Comparing these figures with the minute-volume at complete rest, it is found that these normal subjects could increase their pulmonary ventilation on an average of 10.7 times above the resting value. This gives a fairly accurate idea as to the great adaptability of the respiratory

[4] Barcroft: The Respiratory Function of the Blood. Cambridge, 1914, p. 239.

mechanism to any demands that may be put on it. and one has a quantitative value for what we may call the "pulmonary reserve." Since the high minute-volume depends on an ability to increase the rate and especially the depth of respiration, it is not surprising to find a close relation between the highest minute-volume and the vital capacity, or the volume of air which can be expired after the greatest possible inspiration. There is also a relation between the volume of the individual respiration and the vital capacity, and it is rather striking that the deepest respirations while riding averaged only 33 per cent of the vital capacity. Curiously enough no definite differences were observed with regard to the respiratory mechanism between the trained and the untrained subjects. A point of considerable interest was the great difficulty experienced in making the subjects highly dyspneic, because they tended to stop riding on account of muscular fatigue rather than on account of shortness of breath. This was in part due to the fact that they were using muscles unaccustomed to heavy work, but it showed that in general the respiratory mechanism can normally adapt itself to any grade of metabolism that the body can produce.

Normally then, "the pulmonary reserve" is so great, and the minute-volume of air breathed can be so easily raised to many times the volume at rest, that dyspnea is only noticeable under conditions of rather severe exertion. What, however, are the factors which tend to decrease the "pulmonary reserve" or to make a person more readily subject to dyspnea? What must one consider as possible elements in the cause of any pathological dyspnea? Since the "pulmonary reserve" depends on the relation between the minute-volume of air breathed at rest and the highest minute-volume which the subject is capable of breathing, it will be greatest if the minute-volume at rest is low. Thus, first among the factors which may cause an abnormal tendency to dyspnea are those conditions which produce a high minute-volume at rest. Chief among these are an increase of metabolism and the presence of an acidosis. Secondly there are the factors which limit the ability of the subject to meet a demand for a higher pulmonary ventilation. Since an increase in minute-

volume depends on an increase of rate and depth of respiration it is evident that a high initial respiratory rate and more especially, anything which interferes with deep breathing will tend to reduce the pulmonary reserve. Finally it will be seen that still other conditions probably underlie the type of dyspnea which is associated with periodic breathing.

From this point of view, then, we may approach the question of heart disease in an attempt to determine whether or not these possible factors are present, and in how far they may be considered as elements in the production of dyspnea. It is important to appreciate that dyspnea is one of the commonest symptoms met with in patients suffering from cardiac disorders, and that it appears in a considerable variety of clinical conditions. We shall, therefore, expect to find that the causes of dyspnea are not necessarily the same in different cases, and that while the symptom has a comparatively simple basis in certain instances, in others it is complex and depends on a number of interacting factors. The dyspnea which is noticed on ascending stairs by a subject with a compensated valvular lesion is quite a different thing from the continuous dyspnea of the same person when in a state of acute decompensation, and this in turn may have different underlying elements from the dyspnea of the patient with cardio-renal disease, or the nocturnal attacks of paroxysmal dyspnea seen in an old man with chronic myocarditis.

Let us first consider the question of the metabolism in cardiac disease. The most satisfactory study of the basal metabolism in patients with heart disease was carried out at Bellevue Hospital, New York, in the calorimeter of the Russell Sage Institute of Pathology by DuBois and Meyer in an investigation in which it was my privilege to take part.[5] Of fundamental importance was the demonstration by means of the close agreement between the methods of direct and indirect calorimetry, as well as by the finding of respiratory quotients which were within the normal limits, that the intermediary metabolism in heart disease follows a normal course. Sixteen patients were studied. The results showed that in compensated cardiac disease the metabolism is

[5] Peabody, Meyer and DuBois: Arch. Int. Med., 1916, xvii, 980.

perfectly normal. Of twelve patients, on the other hand, who had some degree of dyspnea at the time they were studied, three showed a normal metabolism, and nine a metabolism that was distinctly above normal. In five of the latter the metabolism was increased from 25 to 50 per cent above the normal. The cause of the rise in metabolism is not evident. These, and other more recent observations from the same source [6] indicate that it is not a necessary accompaniment of dyspnea, that it cannot be attributed to acidosis, and that it bears no definite relation to the level of the nitrogen in the blood. The subject has been further investigated in the Medical Laboratory of the Peter Bent Brigham Hospital [7] in association with Dr. J. A. Wentworth and Miss B. I. Barker. The indirect method of calorimetry was used, the apparatus consisting essentially of a large Tissot spirometer for the collection of the expired air and the Haldane Portable Gas Analysis apparatus. By this method data are obtained regarding the minute-volume of air breathed which are lacking in the observations made with the large bed calorimeter. The results of the metabolism determinations in 24 instances agree essentially with those at the Sage Institute. They confirm the fact that in persons with mild grades of heart disease, in whom the lesion is comparatively well compensated, the metabolism is within normal limits, and they demonstrate again that in more severe cases, with or without dyspnea at the time of observation, the metabolism is variable, being frequently normal, but in some instances as much as 40 per cent above normal. In only two cases was the heat production more than 25 per cent above the normal however, and in general, the rise in basal metabolism is neither a constant, nor a particularly significant feature. Of more immediate interest in the study of dyspnea are the observations on the minute-volume of air breathed. These show that while patients with mild cardiac lesions, and only a slight tendency to dyspnea breathe a normal minute-volume of air, usually between 5.0 and 6.0 liters, the more severely affected patients who are either dyspneic while at rest, or who become so on very slight

[6] Aub and DuBois: Arch. Int. Med., 1917, xix, 865.

[7] Peabody, Wentworth and Barker: Arch. Int. Med., 1917, xx, 468.

exertion, tend to have a considerably higher minute-volume. In this group of subjects the minute-volume at rest ran as high as 11.6 liters while the average in 12 patients was 8.22 liters. There is, moreover, no definite relation between the minute volume and the metabolism, and a high minute-volume may be found in a subject whose basal metabolism is wholly normal. A similar increase in the minute-volume has been reported by Beddard and Pembrey [8] and by other observers.

As to the cause of this increased minute-volume associated with a normal metabolism we have no absolute proof, but there is a very suggestive relationship between the raising of the minute-volume and the decrease of the vital capacity of the lungs. Practically all cardiac patients with a vital capacity of less than 60 per cent of the normal (see below) show a high minute-volume and a similar observation has been made in a case of pleural effusion. The decrease in the vital capacity of the lungs is probably associated with a lessening of the area of the respiratory surface, as for instance, by the production of atelectasis by collections of fluid in the pleural cavity. The dead space, consisting of the naso-pharynx, trachea, and bronchi, would not necessarily be affected and the resulting decrease in the respiratory surface, with a relative increase in the dead space would bring about a rise in the actual minute-volume of air breathed in order that the alveolar ventilation, which is after all the essential thing, should remain constant.

In patients with severe manifestations of cardiac disease, then, an increase of the minute-volume of air breathed while at rest is very commonly present, whether or not there is any associated rise in the basal metabolism. In such cases the high initial minute-volume will be a factor in the production of dyspnea in that it limits the "pulmonary reserve." By diminishing the difference between the volume of air breathed at rest, and the maximum volume the subject is capable of breathing, it makes him more readily susceptible to the production of dyspnea.

Let us turn to the consideration of a second condition which causes an increase in the pulmonary ventilation, and which may

[8] Beddard and Pembrey: Brit. Med. Jour., 1908, ii, 580.

thus act as a factor in the production of dyspnea in much the same manner as an increased metabolism. This is acidosis. The respiratory centre is excessively sensitive to a shift in the reaction of the blood, and any considerable accumulation of acids in the blood stream causes a greater activity on the part of the lungs. Indeed the production of hyperpnea is perhaps the most characteristic effect of acidosis.

In the recent enthusiastic attention which clinicians have accorded to the subject of acidosis, the condition has been held responsible for a great variety of symptoms. It is not to be wondered at, then, that the relation of acidosis to dyspnea is a problem which has given rise to much conjecture and to a considerable amount of experimentation. Some observers, notably Lewis and his co-workers [9] regard acidosis as one of the chief factors in the dyspnea seen in elderly persons with weak hearts and usually with kidney involvement,—essentially the cardio-renal group. It is important therefore to examine in some detail into the conditions associated with cardiac disease in which acidosis is present, and to consider in how far it may be regarded as responsible for the production of dyspnea.

As regards pure cardiac disease, one may state as the result of many observations on the carbon dioxid content of the blood and alveolar air, that there is no evidence indicating the presence of an acidosis in compensated cases. In patients with pure cardiac disease in a state of acute decompensation the question is less simple to answer. Not infrequently the alveolar air analyses show a low carbon dioxid tension, while the blood analyses show a normal or high tension. With the regaining of compensation and usually with the disappearance of continuous dyspnea, so that the patient is comfortable while at rest, the alveolar carbon dioxid rises quickly and the relation between the blood and the alveolar carbon dioxid becomes normal. How is this to be interpreted? It is possible that in these acutely sick persons the samples of alveolar air are not reliable, but this explanation is hardly satisfactory, and it is much more likely that the condition

[9] Lewis, Ryffel, Wolf, Cotton and Barcroft: Heart, 1913, v, 45.

is a real one. Peters[10] who has studied the question at the Presbyterian Hospital and who has found the carbon dioxid content of the blood considerably higher than that of the alveolar air, concludes, and most probably correctly, that there is an interference with the passage of carbon dioxid from the blood into the alveolar air. There is thus an accumulation of carbon dioxid in the blood, and an acidosis in which an excess of carbon dioxid is the essential feature. The possibility of the presence of other abnormal acids due to incomplete oxidation, a condition similar to the acidosis of asphyxia, cannot be definitely excluded, but at any rate, in the production of dyspnea in pure cardiac disease acidosis is a factor which only occurs in the most severely decompensated cases, and its influence in cases which recover is of short duration.

In cases of cardiac disease associated with renal insufficiency, on the other hand, the rôle played by acidosis is much more significant. Sellards[11] and Palmer and Henderson[12] showed the frequency with which acidosis occurs in chronic nephritis, and Straub and Schlayer[13] described the low alveolar carbon dioxid tension in uræmia. Observations in our own laboratory have confirmed this work and helped to indicate the close relationship between acidosis and renal function.[14] In general, cases of chronic nephritis with a normal phthalein output show no signs of acidosis; with the failure to excrete phthalein satisfactorily an acidosis develops which shows itself by an increase in the "alkali-tolerance test;" and when the phthalein output has fallen to zero, there is often a degree of acidosis sufficient to cause a fall in the carbon dioxid tension of the alveolar air. The recent work of Marriott and Howland[15] shows that the acidosis is due to the inability of the kidney to excrete acid phosphate.

A study of numerous cases of renal and cardio-renal disease

[10] Peters: Am. Jour. Physiol., 1917, xliii, 113.

[11] Sellards: Bull. Johns Hopkins Hosp., 1912, xxiii, 289; ibid. 1914, xxv, 141.

[12] Palmer: Med. Communicat. Mass. Med. Soc., 1913, xxiv, 133.

[13] Straub and Schlayer: Munchen. med. Wchnschr., 1912, lix, 569.

[14] Peabody: Arch. Int. Med., 1915, xvi, 955.

[15] Marriott and Howland: Arch. Int. Med., 1916, xviii, 708.

shows that in the advanced stages, before and after the onset of uræmia, and even just before death, the alveolar carbon dioxid tension is usually not below 25 mm. This is in itself not a sufficient drop to cause a marked hyperpnea. Indeed in diabetes the increase in ventilation due to acidosis is not particularly noticeable until the carbon dioxid tension is approximately 15 mm. Considering, therefore, the comparatively mild grade of acidosis usually met with in chronic nephritis, one must hesitate to attribute to it too great a significance in the production of dyspnea. Occasional rare cases of nephritis present the clinical picture of coma and air hunger just before death, and simulate diabetic coma. In these the carbon dioxid tension is about 10 mm., and the air hunger may be relieved by alkali. Thus in a very small group of cases the acidosis may be the direct cause of a hyperpnea which is sufficient to produce dyspnea.

If, however, the acidosis which is commonly met with in chronic nephritis is not of itself intense enough to cause dyspnea, it is by no means true that it is a factor to be ignored. Its significance may be made clear by some experiments carried on at the Peter Bent Brigham Hospital [16] which were devised as a means of studying the production of dyspnea in normal subjects and in persons with cardiac disease. In order to avoid the dangers and difficulties attendant on the production of dyspnea in persons with heart disease by exercise, and to allow of the investigation of comparatively sick patients in bed, the dyspnea was produced by a continually increasing percentage of carbon dioxid in the inspired air. The subjects breathed through valves separating the inspired from the expired air. The expired air passed through a plethysmograph which was calibrated so that its movements, recorded on the smoked drum of a kymograph, gave an accurate index of the volume of each respiration as well as of the rate of respiration. The total ventilation for each minute could thus be calculated. After leaving the plethysmograph the expired air was rebreathed by the subject. The carbon dioxid tension of the inspired air rose progressively during the experiment and its percentage was determined by the analysis of samples taken at

[16] Peabody: Arch. Int. Med., 1915, xvi, 846.

frequent intervals. As the result of a series of observations it was found that in normal individuals a given percentage of carbon dioxid produced a fairly constant rise in the pulmonary ventilation. Thus when the inspired air contained from 4.2 to 5.4 per cent of carbon dioxid the minute-volume of air breathed was approximately twice what it was at the beginning of the experiment. Exactly the same relationship was observed in most patients with cardiac and renal disease. Their response to carbon dioxid fell into the normal limits. In a number of cases, however, in which the alveolar air showed evidence of an acidosis, abnormal findings were met with. Instead of the pulmonary ventilation being doubled by 4.2 to 5.4 per cent carbon dioxid it became doubled when only 2 to 3 per cent of carbon dioxid was breathed. In other words these patients were unusually sensitive to the stimulus of carbon dioxid, and it required much less than normal to cause a considerable increase of the pulmonary ventilation. That this effect was actually dependent on the acidosis was demonstrated by performing the experiment again after enough alkali had been given to overcome the acidosis and to bring the carbon dioxid tension of the alveolar air back to its normal value. Under these circumstances the patients reacted just like normal subjects. The explanation of these results is simple. With the development of the acidosis the so-called "buffer action" of the blood becomes diminished and the addition to it of small amounts of carbon dioxid which under normal circumstances would produce little change in reaction, causes enough shift in reaction to stimulate the respiratory centre. It seems fair to conclude from these experiments that while the degree of acidosis which is commonly met with in patients with cardio-renal disease is not sufficient to cause any decided increase in the pulmonary ventilation, nevertheless it may render the patients unusually susceptible to the production of dyspnea, and it is to be regarded as one factor in causing them to become short of breath on exertion. In severely decompensated cases, even the comparatively slight increase in the pulmonary ventilation while at rest may be sufficient to make the difference between comfort and discomfort in breathing. There is then a rational

basis for the administration of alkali to patients with acidosis, and in certain cases definite relief of symptoms may be observed.

Having discussed briefly the two chief conditions which cause an increase of the pulmonary ventilation let us now turn to the means by which the body responds to a demand for a higher minute-volume of respired air, and consider in what way these may be affected in heart disease. Such an increase in the minute-volume of air breathed is brought about by an increase of the rate or of the depth of breathing.

We may first give attention to the question of the depth of respiration and observe in how far a limitation in the capacity to breath deeply is to be regarded as a factor in the production of dyspnea in heart disease. In the experiments just described in which the subjects were made dyspneic by rebreathing air containing increasing amounts of carbon dioxid, one striking difference was noted between the normal subjects and the patients who had cardiac disease.[17] While the former did not become extremely dyspneic until they were breathing from 60 to 80 liters of air per minute, the latter were forced to stop when they were breathing only 20 to 40 liters per minute. A study of the graphic records of the respiration during the experiments showed that this difference depended on the fact that the patients with cardiac disease were unable to increase the depth of their respiration as well as the normal subjects could. It is obvious that anything which prevents a person from breathing deeply is of profound importance as a factor in the production of dyspnea, for it immediately limits the extent to which the minute-volume can be raised, and this prevents him from meeting such increases of metabolism as he normally could. The inability to breathe deeply was found to correspond to a decrease in the vital capacity of the lungs.

It has long been known [18] that the vital capacity of the lungs is often decreased in heart disease, but no particular attention has been paid to the fact. It seemed, however, that the condition merited systematic investigation, and in association with Dr. J. A. Wentworth a careful study of the subject has been

[17] Peabody: Arch. Int. Med., 1917, xx, 433.

[18] Arnold: Uber die Athmungsgrosse des Menschen. Heidelberg, 1855.

made.[19] The vital capacity of the lungs is the volume of air that can be expired after the deepest possible inspiration. In our experiments the observations were made by having the subject breathe in and out as deeply as possible through a rubber mouthpiece connected with a calibrated recording spirometer. The movements of the spirometer were recorded on the smoked drum of a kymograph, and the vital capacity was determined by measuring the length of the line which corresponded to the greatest expiration and inspiration. In order to decide whether the vital capacity of any given patient was normal or not, it was necessary to have standards for comparison, and since no wholly satisfactory data were at hand observations were made on a considerable group of healthy persons. Ninety-six normal men and forty-four normal women were studied. It was found that standards which were sufficiently accurate could be established if the results were classified according to sex and according to height. Various other factors which influence the vital capacity of the lungs could be fairly neglected as they were not particularly significant in the group of cases which we have studied. Thus old age causes a decrease in the vital capacity, but the majority of our patients were at a time of life when this did not play an important part. Athletic training increases the vital capacity but this rarely affected our results, for in pathological cases it is the decrease that is significant. When placed in their appropriate groups according to sex and height it was found that 134 of the 140 normal subjects had a vital capacity of 90 per cent or more of the normal figure.

Having thus established normal standards of the vital capacity of the lungs for men and women of different heights, it was possible to compare with them the results obtained in patients with heart disease. One hundred and twenty-four cases have been studied and about 224 records have been made. It is convenient to classify these patients according to the vital capacity into four groups each of which presents rather definite clinical characteristics, and it will be seen that there is a very close relationship between the decrease in vital capacity and the tendency to dyspnea. Briefly summarized the results obtained are somewhat as follows:

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[19] Peabody and Wentworth: Arch. Int. Med., 1917, xx, 443.

TABLE I.

Group	Vital Capacity, per cent.	Number of cases.	Mortality, per cent.	Symptoms of decompensation, per cent.	Working, per cent.
I	90+	25	0	0	92
II	70–90	41	5	2?	54
III	40–70	67	17	39	7
IV	under 40	23	61	100	0

Certain cases were tested several times and, owing to changes in the vital capacity they appear in more than one group. In the "Mortality" column they are included only in the lowest group into which they fell. "Symptoms of decompensation" indicates dyspnea while at rest in bed or on very slight exertion. Under "Working" are included only those actually at work, and able to continue. Many other patients in Group II were able to work, but they are not included as they were still in the hospital.

Group I consists of 25 cardiac patients in whom the vital capacity was 90 per cent or more of the normal standard. Thus in these cases the vital capacity does not fall below the limits found in healthy persons. All of them had well compensated hearts, and dyspnea was scarcely a more prominent symptom in their histories than it would be found to be in a similar group of normal individuals. About 90 per cent of them were working, and the others were limited in their activities by cardiac pain or palpitation rather than by dyspnea. They were thus nearly all in extremely good general condition, and in many the cardiac lesion was merely an incidental finding. Group II consisted of 41 cases whose vital capacity was between 70 and 90 per cent of the normal. These patients differed from those of the group with a higher vital capacity in that practically all gave a definite history of dyspnea on any unusual exertion. The majority, however, were able to work, and the rest, with two possible exceptions, could lead a satisfactory, though somewhat restricted life. Several of them had passed through periods of more or less severe cardiac decompensation, and they are to be regarded as borderline cases whose activities must be somewhat limited, but who, under favorable circumstances, show little evidence of cardiac insufficiency. Group III consists of 67 patients in whom the vital capacity was between 40 and 70 per cent of the normal.

These cases are much more severely handicapped than are the members of Group II and practically all suffer from dyspnea on moderate exertion. Those with a vital capacity only slightly above 40 per cent are confined to bed or can do little more than get about the house, while those with a vital capacity approaching the upper limits can walk fairly easily, but they usually avoid the stairs or hills. Only 7 per cent of this group were still at work. Attacks of severe cardiac decompensation occur with considerable frequency among those patients, and 17 per cent of the number have died. Group IV consists of 23 cases with a vital capacity of 40 per cent or less. All of them were severely decompensated and the majority were confined to bed. Dyspnea is either constantly present or it is produced by the slightest exertion. The prognosis for patients who fall into this group is bad. A few patients whose vital capacity has fallen as low as this during their first attack of decompensation have subsequently recovered so that they could lead a fairly active life, but most of them made comparatively little clinical improvement and 61 per cent have died.

These observations demonstrate the important rôle played by decrease of the vital capacity in the production of dyspnea in heart disease. In a surprisingly accurate manner the degree to which the vital capacity is decreased corresponds to the tendency to dyspnea. Patients who have no unusual tendency to become short of breath almost invariably have a normal vital capacity, and those who become dyspneic readily have a vital capacity which is depressed in accordance with the severity of the symptom.

But what, it may be asked, is the cause of the decrease of vital capacity in heart disease? The answer to this question is that there are many causes, some of which are obvious and easy to appreciate, while others still remain obscure. Anything which interferes with the free movements of the lungs, or the entrance of air into them, will decrease the vital capacity. Thus pleural effusions, fluid in the peritoneal cavity, emphysema and pulmonary oedema may be reckoned among the more gross conditions affecting it. These and other similar factors seem to explain

the more severely decompensated cases, but there is a large group of patients with slight symptoms in whom the physical examination gives no clew to the reason for a decreased vital capacity. Further investigation into the cause of the decreased vital capacity in these subjects is clearly indicated, and the work of Siebeck [20] points to a promising line of approach. His comprehensive study of lung volumes in heart disease suggests that the low vital capacity depends on a change in the elasticity of the lungs which results from an engorgement of the pulmonary vessels due to back pressure from the left side of the heart. If this conception is correct then the vital capacity of the lungs is an index of the state of the pulmonary circulation, and as such is of considerable clinical signifiance. It is probable that in many cases the earliest evidence of cardiac insufficiency occurs in the pulmonary circuit but the usual methods of examination afford no means of detecting it. One clinical fact which is quite in accord with the theory that decrease in vital capacity with its attendant dyspnea is associated with a disturbance of circulation through the lungs is the common observation that dyspnea is an earlier symptom in disease of the mitral valves than it is in disease of the aortic valves.

If this relation between the vital capacity and the tendency to dyspnea is generally true when one compares a large series of cases with somewhat arbitrarily chosen normal standards it becomes more so when one follows the individual patient and watches the changes in the vital capacity which are coincident with changes in the clinical condition. As long as the clinical picture remains constant the vital capacity is found to be the same, but when cardiac insufficiency becomes more marked, and dyspnea more noticeable, the vital capacity falls. Similarly, an improvement in the general condition and a lessening of the dyspnea is associated with a rise in the vital capacity. This parallelism is, indeed, so definite that the determination of the vital capacity seems to assume a practical significance. Dyspnea is, of course, only one symptom of heart disease but it is a very common symptom, and it is an important one because the degree of dyspnea

[20] Siebeck: Deut. Arch. f. klin. Med., 1910, c, 204.

or of the tendency to dyspnea is a valuable index of the state of cardiac efficiency. The clinical records of cardiac patients abound with statements about dyspnea but these are always of limited worth for they are based on either the history as given by the patient or on the gross examination of the physician. Dyspnea is, moreover, such a difficult condition to analyze or to describe, that any objective method which allows one to obtain accurate quantitative information regarding it will serve a useful purpose. Such information the determination of the vital capacity appears to furnish even if only in a somewhat rough way. In many instances the observations have proved to give a more reliable conception of the clinical condition of the patient than has been obtained from either the history or the physical examination. This of course is true only in cases in which dyspnea is the presenting symptom, and does not hold for the group of patients whose cardiac lesion manifests itself by other symptoms such as pain or palpitation. However the latter includes only a relatively small number of cardiac cases, and in a surprisingly large proportion of cases records of the vital capacity give important and helpful data as to the present status and the prognosis. They are often of much greater significance than are records of the pulse rate or blood pressure, and they seem to be a useful, although indirect index of the cardiac reserve.

But, as we have already seen, the increase of the minute-volume of air breathed which accompanies a rise in metabolism is brought about not only by a greater depth of respiration but also by a higher rate of respiration. What, then, is the relation of rate of respiration to the problem of dyspnea in heart disease? The facts are simple and well known by all, so that the subject may be briefly dismissed. With the exception of the extremely mild cases of cardiac disease, which are in a good state of compensation, most instances have a respiration rate which is somewhat above normal, and the more severely affected the case the more rapid the respiration. Now there is, roughly speaking, a maximum rate to which the respiration can rise without losing much of its efficiency. The extraordinarily rapid breathing seen in some hysterical patients, is of course economically wasteful.

The maximum efficient respiratory rate will vary considerably in different individuals and under different circumstances, but it is interesting for purposes of comparison to note that the average highest rate of our normal bicycle riders was 34 per minute. Assuming some such figure as this for the high limit of efficient respiratory rate, it is obvious that the individual with a low initial rate while at rest has a marked advantage. The greater the difference between the rate of respiration at rest and the maximum rate of efficient respiration, the greater is the reserve. With an initial rate of ten the respiration rate can be raised more than three times before the maximum of efficiency is reached, but with an initial rate of seventeen it can only be doubled. Thus the high rate of respiration which is found in severely affected cardiac patients is a significant factor in decreasing their reserve and increasing their tendency to dyspnea.

Having considered some of the general conditions which bear on the problem of dyspnea in heart disease we may now turn to a special type of respiration which deserves mention both because it is common in clinical practice and because its mechanism involves other considerations than those which have been as yet discussed. This is the periodic type of breathing, which reaches its highest expression in the classical Cheyne-Stokes respiration. Careful observation, and more particularly the studying of records made with the pneumograph impress one with the fact that the association of periodic breathing with heart disease is much more frequent than is generally recognized. It appears in cardiorenal cases, in aortic disease, and in advanced myocarditis, and it is most often characteristically seen in patients who suffer from attacks of nocturnal dyspnea. A history of the onset of dyspnea in the evening is often given by patients with myocardial weakness, and if they are watched it will usually be found that periods of dyspnea alternate with periods of apnea. During the apnea the patient dozes off and goes to sleep. With the beginning of respiration he rouses a little, and at the height of dyspnea he wakes up to find himself intensely uncomfortable and often gasping for breath. His discomfort disappears with the cessation of dyspnea, and during the period of apnea he falls

asleep again. Such attacks are sometimes referred to as "cardiac asthma," but the name is singularly ill-chosen for one of the most characteristic features of the true asthmatic attack is that the breathing is continuously rapid and labored. The volume of air expired has been measured in a few cases of mild periodic dyspnea and the total minute-volume has not been found to be remarkably high. The chief difficulty, and the reason for the discomfort appears to be that the patient is breathing only part of the time. The periods of apnea may last for half a minute, so that the patient is virtually breathing his minute-volume in the remaining thirty seconds. If he were to breathe the same minute volume of air regularly, over the whole minute, much less discomfort would be experienced. The volume of the individual respirations rises to much above the normal, and since the vital capacity is usually decreased, the deepest respirations may approach the maximum of which the patient is capable.

What can one say as to the fundamental cause of this type of dyspnea? The question is unfortunately one which remains incompletely answered, but some facts have been gathered which throw light on it. The suggestion has been made that the attacks are associated with an acidosis. As opposed to this it is difficult to conceive of an acidosis of such sudden onset, and moreover the typical feature of the respiration in acidosis, such as that seen in advanced diabetes, is hyperpnea with deep regular breathing. The clinical picture is quite different from that of periodic breathing. However, to settle the problem more definitely Dr. F. T. H'Doubler has studied the carbon dioxid content of the blood in a number of cases during the attack of dyspnea and either before or after it. Some of the patients who had advanced cardiorenal disease showed a slight decrease in the carbon dioxid tension, but this was rarely below 25 mm. and not sufficient to account for the dyspnea. Moreover there was no significant fall in the carbon dioxid tension during the attack of dyspnea as would be expected if the attack were dependent on a further increase in acidosis.

Douglas and Haldane [21] consider that the essential cause of Cheyne-Stokes respiration is oxygen lack, and they state that

[21] Douglas and Haldane: Jour. Physiol., 1909, xxxviii, 401.

"the periodic breathing is produced by periodic occurrence and disappearance of the (indirect) excitatory effects of want of oxygen" which "may be due to abnormal deficiency in the alveolar oxygen pressure" or "to effects on the circulation of changes in the breathing or to both causes combined." This explanation accounted satisfactorily for the periodic breathing observed by them on the expedition to Pike's Peak.[22]

The frequency with which the attacks come on at night is a feature of interest. Periodic breathing is a normal phenomenon which occurs in many healthy persons during sleep, and in hibernating animals. Straub [23] has shown that during sleep the alveolar carbon dioxid tension rises, and he attributes this to a decrease in the excitability of the respiratory center. Morphine, which depresses the respiratory centre, often produces periodic breathing. May it not be that the periodic breathing in heart disease is associated with a change in the excitability of the centre? In favor of this suggestion is its nocturnal occurrence, and the fact that in mild cases it often ceases if the patient is roused or in any way excited. To test the question further, some observations have been made with Mr. F. C. Hall on the effect of caffein, a respiratory stimulant, on Cheyne-Stokes respiration. The number of cases as yet examined is comparatively small but in nearly all a definite, though very transient cessation of the periodicity of the breathing, often associated with subjective improvement, resulted from the administration of considerable doses of caffein. Several other drugs produced no noticeable effect. Morphine, in the few instances studied, caused no change or increased the periodicity, but its administration was therapeutically beneficial, for it depressed the central nervous system so that the patients did not rouse during the periods of dyspnea.

Whether Cheyne-Stokes respiration and periodic dyspnea in heart disease are due to oxygen lack in the sense of Douglas and Haldane or to a depression of the excitability of the respiratory

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[22] Douglas, Haldane, Henderson and Schneider: Trans. Royal Soc. London, 1912, cciii, Series B, 185.

[23] Straub: Deut. Arch. f. klin. Med., 1915, cxvii, 397.

centre, or possibly to a combination of the two, is a problem which still awaits solution.

Such then, are at least some of the factors which contribute to the cause of that common but singularly complex symptom of heart disease—dyspnea. In its final analysis the problem resolves itself into the question of what we have called the "pulmonary reserve." The degree to which any individual manifests a tendency to dyspnea depends on the relation between the volume of air which he breathes while at rest and the maximum volume which he is capable of breathing. The ability to meet adequately the needs of an increased metabolism such as occurs with muscular exercise, depends on the "pulmonary reserve." In normal persons, as has been seen, the "pulmonary reserve" is great, and healthy young men can increase their pulmonary ventilation to approximately ten times the volume required by their resting metabolism. But in patients with heart disease the circumstances are much less favorable, and various conditions arise which cut down the "pulmonary reserve" and make them more readily subject to dyspnea. An increase of metabolism, or the development of an acidosis may raise the volume of air breathed while at rest, while an increased respiratory rate or a decrease of the vital capacity of the lungs will make the maximum ventilation of which they are capable much lower than the normal. A decrease of the "pulmonary reserve" results, and even moderate exertion causes a rise of metabolism and a pulmonary ventilation which produces the subjective sensation of dyspnea. The degree to which these different factors are present in any given case is extremely variable. The earliest and most constant feature in the production of dyspnea is apparently a fall in the vital capacity and it is often met with quite unaccompanied by any of the other factors which we have considered. In advanced cases of cardiac disease the situation becomes much more complicated. The vital capacity drops still lower, the rate of respiration rises, the metabolism increases, and an acidosis may appear. Finally the picture is still further confused by the onset of periodic respiration, and it becomes, indeed,

quite impossible to determine which element is most responsible for the patient's unhappy state.

Our conception of the etiology of dyspnea in heart disease is still vague and incomplete. Some little insight we have obtained, but further knowledge must come from the careful investigation of the individual case, the discovery of other factors in the cause of dyspnea, and the systematic grouping of the separate types of dyspnea. Only by such studies can we hope to reach our ultimate aim—the proper treatment and the relief of dyspnea in heart disease.

THE COAGULATION OF BLOOD

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THE clotting of blood takes place, according to the great majority of observers, in two separate stages or phases—First, the formation of thrombin from some antecedent substance existing in the blood. Second, the action of this thrombin on fibrinogen whereby fibrin is formed and deposited as a gel or clot. Two prominent investigators, Wooldridge and Nolf, have dissented from this view, particularly as regards the second stage, but the evidence in its favor is so conclusive that we are justified in accepting it as a basis for a discussion of the details of the process of coagulation. It is convenient to consider the final phase first—The demonstration that fibrin is formed by a reaction between thrombin and fibrinogen we owe to the investigations of Schmidt and of Hammarsten. In his numerous researches upon the coagulation of blood Schmidt [1] laid the foundation for all subsequent work. The summary of these investigations presented in his book contains a wealth of significant observations. Some of these have been amplified and elaborated by later workers, but there are some that as yet have not been followed out by modern methods. They offer leads for promising .investigations. Schmidt's final theory of the process of clotting need not be described, since in some respects it has been made untenable by later work. But we owe to him the conclusive demonstration of the existence of thrombin, and of its essential rôle in the final act of clotting. His work was supplemented by the careful experiments of Hammarsten.[2] This observer proved that the substance acted upon by thrombin is fibrinogen, a globulin that exists normally in the circulating blood.

Making use of the methods discovered by these investigators, or of the improvements upon these methods suggested by later workers, it is a simple matter to prepare separately these two

substances in approximately pure condition, free at least from admixture with other so-called fibrin factors. When thus prepared the addition of the solution of one to that of the other, in proper proportions, is followed in a few minutes by the gelatinization or clotting of the mixture. For the convenience of other workers I may describe briefly the methods that I have found most effective in the preparation of thrombin and fibrinogen.

Thrombin is prepared from freshly formed fibrin—Blood from slaughter house animals (I have used always pig's blood) is defibrinated by whipping with the hand. The strings of fibrin thus obtained are washed in cold water, with constant kneading and pulling, until the hemoglobin is removed. The white mass of fibrin is squeezed dry, minced with scissors and then covered with an 8 per cent solution of sodium chloride. The solution is kept in a refrigerator for 48 hours and is then filtered through cheesecloth. The somewhat viscous filtrate should be rich in thrombin. It can be tested by adding a drop or two to a fresh solution of fibrinogen or to some oxalated plasma. The preparation may be preserved in this crude form or it may be further purified. If preserved in the crude form the excess of sodium chloride should be reduced to about 1 per cent by dialyzing in a collodion tube against seven times its volume of distilled water. The material in the tube is then filtered, distributed in lots of one or two cc. in watch crystals and evaporated to dryness in a current of air from an electric fan—the watch crystals are kept in a desiccator until needed. If a purer preparation of thrombin is desired the first filtrate from the digested fibrin is precipitated by the addition of an equal volume of acetone. The mixture is thrown on a series of small filters, 25 to 50 cc. to each filter, and allowed to filter completely. Each filter is then opened, the precipitate on the paper is spread as thinly as possible with a spatula and the papers, pinned to a board, are dried quickly before an electric fan. The dry filter papers are kept for a day or so in a desiccator and are then extracted with water by placing them in a flat dish and covering with distilled water—They are allowed to extract for an hour, without stirring, the water is then filtered off—the filtrate should be clear. This filtrate may be distributed in small lots in watch crystals, dried quickly and preserved in a desiccator. Some small traces of coagulable protein are still found in this preparation. This impurity may be got rid of by a second precipitation with acetone, the precipitate being treated as above, or by shaking once or twice with chloroform and filtering. Both of these latter procedures are accompanied by a loss of thrombin, but they give a final preparation that is free of all traces of coagulable protein.

Fibrinogen.—Fibrinogen solutions do not keep well. It is advisable as a rule not to use them for more than 24 to 48 hours—for this reason

it is better to prepare it in small lots at a time and the blood of the cat furnishes a convenient source. The method of preparation that I have employed is a slight modification of that devised by Hammarsten, the chief difference being the use of the centrifugal in sedimenting the precipitate in place of filtration. The animal after fasting for 24 hours is anesthetized with ether and bled from the carotid through a paraffined cannula into an oxalate solution, consisting of 1 per cent of sodium oxalate made up in 0.9 per cent sodium chloride, boiled and filtered. The oxalate solution is used in the proportion of 1 to 8 of the blood. The tubes in which the blood is caught are inverted to mix the contents and are then centrifugalized at high speed for 20 minutes—the clear plasma is drawn off—to this plasma one adds enough of a saturated solution of sodium chloride to produce a good precipitate—usually an equal volume or somewhat more of the salt solution. The mixture is placed at once in centrifugal tubes and centrifugalized at high speed for 5 minutes. The supernatant liquid is poured off, the tubes are drained and rinsed once carefully with a little of a half saturated solution of sodium chloride. The sediment is then covered with some of the half-saturated solution for a few minutes. This solution is poured off, the tube is wiped out with filter paper and the sediment is dissolved in a 2 per cent solution of sodium chloride with stirring. This solution is filtered and again precipitated by the addition of an equal volume of a saturated solution of sodium chloride. This precipitate is then centrifugalized, washed, dissolved and filtered as in the case of the first precipitate, except that in the final solution a 1 to 1.5 per cent solution of sodium chloride is used. Two precipitations suffice, if attention is paid to the washing, to obtain a solution of fibrinogen that clots readily with thrombin, but does not clot spontaneously nor after the addition of calcium chloride or calcium chloride and tissue extract (Cephalin).

Is Thrombin an Enzyme?—Schmidt believed that thrombin belongs to the group of enzymes or ferments. In accordance with the prevalent conception of enzyme action this belief implies that thrombin does not take direct part in the formation of fibrin. Acting as a catalytic agent it induces directly, or through an intermediate reaction, the conversion of all or a part of the fibrinogen to fibrin. Schmidt based his belief upon the two reactions generally considered as characteristic of enzymes—namely their thermolability and the fact that they are not destroyed in the reaction they cause. In regard to the former it is quite true that thrombin in its normal environment, in blood plasma or serum, is destroyed by heating to 60° C. for even a

short time. But experience has shown that this is not the case under all conditions. Rettger [3] showed that an aqueous solution of thrombin prepared according to Schmidt's method will stand boiling for several minutes without completely losing its power to act upon fibrinogen—indeed, Schmidt himself called attention to this fact. Subsequent observations reported by myself [4] indicate that this result is owing to the absence of inorganic salts in such solutions or rather to their low concentration. In dilute aqueous solutions of thrombin made by my method and dialyzed thoroughly against distilled water, boiling for five minutes or more while it weakens the action of the thrombin does not destroy it entirely. When to the same solution one adds sodium chloride to a concentration of 0.5 to 1 per cent boiling for a minute destroys the thrombin completely so far as its action on fibrinogen is concerned. While this fact is interesting it is not determinative in regard to the enzyme character of thrombin. It is known that the enzymes vary rather widely in regard to what may be called their lethal temperature, and that this temperature may be much influenced by the presence of other substances. Whether or not the absence of inorganic salts influences the thermolability of any of the typical enzymes in a manner similar to that observed with thrombin has not been determined, so far as I know.

In regard to the second and more significant property of enzymes, namely, that they act upon their respective substrates after the manner of catalytic agents, the evidence at hand indicates that thrombin does not exhibit this characteristic. The reaction between thrombin and fibrinogen appears to be rather of a fixed or quantitative character in the sense that a given amount of thrombin converts only a definite or limited amount of fibrinogen to fibrin. Thus Rettger was able to show that the amount of fibrin obtained from a given solution of fibrinogen increases with the amount of thrombin added.

For example:

5 drops of thrombin yielded 0.2046 grms. of fibrin
10 drops of thrombin yielded 0.3573 grms. of fibrin
20 drops of thrombin yielded 0.6089 grms. of fibrin
40 drops of thrombin yielded 1.5872 grms. of fibrin

In some later experiments I attempted to obtain more definite results by determining the actual weight of thrombin entering into the reaction.[5] A solution of purified thrombin was used and the weight of thrombin was estimated as the difference between the weight of the residue after evaporation and the same residue after incineration, on the assumption that all the organic matter present was thrombin. The experiments gave figures of this kind:

0.05 mgm. of thrombin yielded 10.75 mgm. of fibrin
0.16 mgm. of thrombin yielded 34.00 mgm. of fibrin
0.25 mgm. of thrombin yielded 36.80 mgm. of fibrin
0.64 mgm. of thrombin yielded 42.50 mgm. of fibrin

Other observers have also noted the fundamental fact that a submaximal quantity of thrombin allowed to act upon a solution of fibrinogen converts only a part of the fibrinogen to fibrin no matter how much time is permitted for the reaction to take place. In view of these results it would seem to be necessary to assume either that the thrombin unites with the fibrinogen in some definite way, or else that in the course of the reaction the formation of the fibrin inhibits in some way the further activity of the thrombin. One might suppose for example that the fibrin as it forms adsorbs the thrombin and thus removes it from the possibility of further action. It is not possible at present, so far as I can see, to come to any satisfactory decision in regard to this point. As stated below there is little or no evidence that the fibrinogen undergoes any profound chemical change in its conversion to fibrin. The change seems to be rather of a physical character in that the fibrinogen is aggregated out of its colloidal solution in somewhat the same way as certain colloids may be aggregated out by the action of inorganic salts. But in this particular case the fibrin-aggregates, if I may use this term, are different in structure and properties from fibrinogen-aggregates produced by other reagents. It would seem quite possible or probable that these fibrin-aggregates represent in fact a real physico-chemical combination between fibrinogen and thrombin.

Specificity of the Thrombin.—As far as we know the reaction between fibrinogen and thrombin is specific in the sense that thrombin has no coagulating action upon other proteins and on

the other hand no agent other than thrombin is capable of converting a fibrinogen solution to a fibrin gel. In this particular the action of the thrombin is highly specific, as in the case of the enzymes, but on the other hand there is little or no evidence of any specificity of action in relation to the different species of animals. In my own work it has happened that thrombin has always been prepared from pig's fibrin, but this thrombin acts apparently with equal facility upon the fibrinogen or the blood plasma of other mammals, or indeed of other vertebrates if one may generalize from experiments made with the blood of the bird and the terrapin. Throughout the vertebrates apparently the fibrinogen is sufficiently uniform in properties to give the characteristic fibrin gel with pig's thrombin, and presumably the thrombin from any other animal would exhibit the same universality in its action.

Nature and Properties of Thrombin.—The thrombin as prepared by Schmidt's method is very far from being pure. While its aqueous solutions may give no precipitate on boiling, owing to the small concentration in salts, the addition of some neutral salt will cause the formation of a relatively large precipitate on boiling, indicating the presence of considerable amounts of a foreign protein. In the two methods that I have used for the isolation of thrombin [6] I have succeeded in obtaining preparations that were free at least from coagulable protein. Such preparations gave no precipitate nor opalescence upon boiling with or without the addition of inorganic salts, but they exhibited positive protein reactions; for example, they gave the biuret, the tryptophan and the Millon's reactions, and addition of ammonium sulphate to one-half saturation threw down a precipitate which on resolution showed marked thrombic action.

When solutions of this purified thrombin were shaken repeatedly with chloroform they finally ceased to give detectible protein reactions, but at the same time they ceased to show any thrombic action, so that one must conclude either that the thrombin itself is a protein, soluble in water and not coagulated by boiling, or else that it is so closely associated with a protein of this character that it is not possible to separate them by any

method yet suggested. My own opinion is that thrombin is a protein or protein derivative. Its tryptophan reaction is especially distinct and this fact would suggest that the indol grouping is characteristic of and perhaps essential to its structure. Rettger called attention to the fact that putrefaction does not readily destroy thrombin, indeed often seems to increase its activity, and this observation suggests the possibility that there may be an essential grouping, containing probably indol, which is responsible for the thrombin action, but that this nucleus may be combined in the blood in some larger complex, and that consequently different methods of preparation of thrombin may yield products exhibiting somewhat different properties and activity. The older view advocated by Pekelharing[7] that thrombin is a nucleo-protein is not borne out by my preparations. The purified thrombin gives no reaction for phosphorus.

Origin of Thrombin.—Thrombin as such is probably not a normal constituent of the blood or of any of the body tissues. It is possible of course that it may occur in traces in the blood at times, but if so it is promptly removed or combined. In effective concentrations it occurs only under such abnormal conditions as lead to the clotting of blood. What does exist in the blood is a mother substance or antecedent material for which the names of prothrombin, thrombogen, proserozym, etc., have been proposed. In discussing the origin of thrombin therefore what we are really concerned with is the origin of the prothrombin. The earlier speculations upon this point were confused by a failure to distinguish between this essential substance and the accessory substances contained in the tissues, or between this substance and a second source of thrombin found in the serum after coagulation and known as metathrombin. The work of Schmidt revealed the difference between thrombin and the zymoplastic material of the tissues, and the work of Morawitz cleared up the confusion in regard to prothrombin and metathrombin. Prothrombin itself is pictured as a substance constantly present in normal plasma, which is converted into active thrombin in the changes preceding coagulation. Morawitz has furnished good evidence for the view that one source at least of this prothrombin

is the blood platelets.[8] These elements when obtained in quantity by differential centrifugalization yield on solution in water a substance which by itself will not coagulate fibrinogen, but which can be made to furnish active thrombin when submitted to the action of calcium salts. The results obtained by Morawitz have been corroborated in my laboratory by Bayne-Jones.[9] There is a bare possibility that in these experiments the prothrombin was simply adsorbed by the platelets, since it has been shown that prothrombin, like thrombin, is readily adsorbed by precipitates such as calcium fluoride or calcium oxalate,[10] but the greater probability is that the prothrombin is a constituent of the blood platelets, and that when these fragile elements dissolve in the plasma they yield to it some prothrombin. At the time of the shedding of blood there is a massive destruction of platelets which must add a large increment to the supply of prothrombin carried by the circulating plasma.

The more or less complete examinations made by various observers to detect the presence of prothrombin in other tissue elements have given either negative or uncertain results with the exception of an interesting series of observations made in my laboratory by Drs. C. K. and K. R. Drinker.[11] In these experiments the bone marrow of the tibia of a dog was perfused through its nutrient artery with solutions of sodium chloride 0.9 per cent, or with a Ringer's solution. It was found that the marrow gives a large yield of prothrombin when perfused with solutions of sodium chloride, and a yield of active thrombin when Ringer's solution is used, since in this case the calcium of the Ringer's mixture serves to activate the prothrombin to thrombin. We must believe, therefore, that the marrow constitutes an important source of production of prothrombin, and since there is much histological evidence to show that the marrow tissue gives rise to blood platelets it may be that the prothrombin is supplied to the blood through the latter element. On the other hand, Hurwitz and Drinker[12] have shown that in rabbits treated with subcutaneous injections of benzol until the marrow is rendered aplastic, there is a marked decrease in the content of prothrombin in the blood together with a decrease in the number of platelets. So

far as our knowledge goes we must consider the bone marrow as the main source of prothrombin, but whether it is given off from this tissue in solution or is passed out in the substance of the platelets to be eventually given to the plasma when these latter dissolve cannot be determined from the evidence at hand. In regard to the chemical nature of prothrombin, and the difference between it and thrombin we practically know nothing at all. I have described a method of separating prothrombin from blood-plasma [13] but not in a form sufficiently pure for chemical study.

Preparation of Prothrombin.—The method that I have used to separate prothrombin from blood-plasma while it does not give the substance in pure form does yield a preparation that is useful for experimental and demonstrational work. It is as follows:

The blood of a cat is oxalated as described above for the preparation of fibrinogen and the clear plasma is obtained by centrifugalizing. The plasma is heated to 54° C. in a water-bath to precipitate the fibrinogen, and filtered. The filtered plasma is precipitated in lots of 5 cc. by the addition of an equal volume of acetone. The precipitate is thrown at once on a filter and the filtration is effected as rapidly as possible by a water pump. The precipitate is washed quickly with ether with the aid of the water pump, and the filter is then removed, the precipitate is spread as thinly as possible with a spatula, dried before an electric fan and kept in a desiccator. When needed one of these papers is cut into small pieces and extracted for ½ hour to 1 hour with 10 cc. of distilled water to which has been added 4 or 5 drops of a 0.5 per cent solution of sodium bicarbonate. The filtered solution when added alone to fibrinogen does not cause clotting, but if calcium chloride is also added the fibrinogen clots in from 5 to 10 minutes. Two drops of a 0.5 per cent solution of calcium chloride may be added to 5 drops of the solution of prothrombin.

The Nature of the Reaction Between Thrombin and Fibrinogen.—Schmidt believed thrombin to be an enzyme or ferment and interpreted its action upon fibrinogen in the light of what was known concerning the action of the more familiar digestive enzymes. This view was adopted in the beginning by Hammarsten [14] after he had shown that fibrin is formed by a reaction between thrombin and fibrinogen. He believed that the fibrinogen underwent hydrolytic cleavage with the formation of fibrin on the one hand, and a soluble protein, fibrin-globulin, on the other. This latter protein was found in the serum. It is a globulin that has

a temperature of heat coagulation of 65° to 66° C., and like fibrinogen is salted out of its solution by half saturation with sodium chloride. Subsequently Hammarsten abandoned this view because quantitative determinations revealed the fact that variable amounts of fibrin, from 61 to 94 per cent, are formed from a given weight of fibrinogen.

Schmiedeberg [15] adopted the cleavage theory and expressed the reaction in the following equation:

$$\underset{\text{Fibrinogen}}{2(C_{111}H_{168}N_{30}SO_{35})} + H_2O = \underset{\text{Fibrin}}{C_{108}H_{162}N_{30}SO_{34}} + \underset{\text{Fibrin-globulin}}{C_{114}H_{176}N_{30}SO_{37}}$$

According to this equation about 48 per cent of fibrin should be obtained from a given weight of fibrinogen, and Heubner in fact stated [16] that when fibrinogen solutions are coagulated by heat (58° to 60° C.) the yield in coagulated fibrinogen constitutes from 48.3 to 49.7 per cent of the fibrinogen used. In some later experiments by Huiskamp [17] it is stated that a fibrinogen may be prepared by precipitation with sodium fluoride, which when heated to 55° C. gives the usual heat coagulum, but without any evidence of a second protein corresponding to the fibrin-globulin. He concluded, therefore, that the fibrin-globulin found in serum exists preformed in the plasma and is not a cleavage product of the fibrinogen formed during coagulation. Hammarsten for the reason stated above abandoned the cleavage hypothesis and suggested that the conversion of fibrinogen to fibrin consists in a molecular transformation of the former, and that the fibrin-globulin represents a variable fraction of the fibrinogen which has undergone incomplete transformation. Nothing has been added to our knowledge of this subject in recent years, and it is quite evident that the whole matter of the quantitative relations of the fibrinogen and fibrin needs reinvestigation, although it is probable that the matter cannot be studied successfully until a method is devised for obtaining pure fibrinogen. In the method used at present we have no guaranty that the fibrinogen obtained is free from other globulins. If, for example, Huiskamp is correct in the supposition that fibrin-globulin exists in the plasma of the circulating blood then it would probably be precipitated

along with the fibrinogen by half saturation with sodium chloride. If the fibrinogen were obtained entirely pure it is quite probable that the yield of fibrin instead of representing from 61 to 94 per cent of the fibrinogen might in fact amount to 100 per cent. The ultra-microscopic observations described below suggest this possibility. At present we can only say that the older view that fibrin is formed by hydrolytic cleavage of the fibrinogen can not be accepted without further evidence.

The Fibrin-gel.—When fibrin is formed in normal coagulation it is not simply precipitated or aggregated out of a colloidal solution. On the contrary it forms a gel of an interesting and peculiar character. The former idea concerning the structure of this gel was obtained from a microscopic examination of clotted blood. It was supposed that the fibrin is deposited as fibrils or threads which form a network enclosing the plasma and corpuscles, and the solidity of the gel was explained on the supposition that the network forms a honeycomb structure in which the plasma as an internal phase is confined within solid septa of fibrin. In 1885 Schimmelbusch [18] as the result of careful microscopic studies stated that the fibrin in clotting is deposited as separate needles of a thin spindle shape and 5 to 20 micra long. This observation was not corroborated, apparently, by later workers and appears to have been forgotten. In 1914 Stübel [19] making use of the dark field illumination described again the formation of fibrin needles or crystals, and in the same year I confirmed his observations in some independent work done with the ultramicroscope.[20] I employed what is known as the slit form of the ultramicroscope. Using solutions of purified thrombin and fibrinogen, or, in place of the latter the clear centrifugalized oxalated plasma, one can obtain easily a beautiful demonstration of the formation of the fibrin needles. The observation cell is first filled with normal saline (NaCl 0.9 per cent) and this is displaced by a suitable mixture of the fibrinogen and thrombin, the concentration of the latter being chosen so as to cause coagulation in a conveniently short time of five to ten minutes. When fibrinogen is used the sequence of events is as follows: The solution shows at first a cone of light in which no particulate structure

Fig. 1.—The crystalline gel of fibrin as seen with the ultramicroscope—some of the fibrin-needles are seen clearly, others are out of focus and blurred. The preparations were made from the oxalated centrifugalized plasma of dog's blood made to clot by addition of a solution of thrombin.

is apparent although it may contain scattered coarse particles. After a certain period varying with the concentration of thrombin used the cone of light becomes more intense, the whole field shimmers with scintillating points and a mass of particles appear that are formed apparently in the previously homogeneous field of the cone of light. These particles assume quickly the shape of short rods that exhibit very active movements which take them into and out of the focal plane, so that continuous observation of a single rod becomes difficult or impossible. The rods lengthen to needles that show less movement as they increase in length and finally the field settles down to a mass of interlaced brilliant needles. When the proportions of thrombin and fibrinogen are not favorable, for example, when the concentration of the thrombin is too low, or when the fibrinogen is in part denatured, the fibrin instead of depositing as firm well shaped needles takes the form of beaded threads or fibers. As regards the size of the crystals it was found in general that the more rapid the coagulation the shorter and finer were the crystals. When oxalated plasma is used in place of the fibrinogen some of the steps described above may be missed. The plasma without the thrombin shows a uniform cone of light in which are many larger particles some of them obviously fat granules. After the thrombin is added the beginning of the process of clotting may be indicated by an intensification of the cone of light and then without obvious disturbance needles appear here and there in the field and rapidly increase in number until they form a stationary intermeshed mass, among which the coarse particles may be seen in Brownian movements. When for any reason the coagulation is very imperfect, as, for example, may result from too small an amount of thrombin or an excess of antithrombin in the plasma, so that the mixture fails to give perhaps a visible clot in mass, one can often observe the formation of scattered fibrin needles that float separately in the field but do not cohere to make a meshwork. Under what may be designated usual or normal conditions the needles are from 10 to 30 microns in length. They appear as separate structures in the irregular mesh that they form, but probably they actually cohere with one another at points of contact.

We may consider the blood clot not simply as a gel but as a crystalline gel. So far as I am aware no similar gel has been observed for any other of the colloids found in living organisms, although examples of crystalline gels more or less similar to that formed by fibrin have been described, especially the gel of barium malonate discovered by Flade.[21]

It would seem from this description that when fibrin acts upon fibrinogen two separate reactions take place—First, the fibrinogen particles, with or without some intermediate change, are aggregated into definite crystalline forms owing to the influence of some directive or vectorial force. Second, the fibrin-aggregate, that is to say, the fibrin needles set up a gelatinization of the system. Under certain conditions the second reaction, the gelatinization may occur without the crystallization. When sodium carbonate for example is added to the plasma so as to produce an appropriate concentration of hydroxyl ions the subsequent addition of thrombin causes the formation of a clot or gel in which no visible structure can be made out with the ultramicroscope. In this case the fibrin-aggregates are formed without doubt and in consequence of their presence the system gels, but these aggregates are not massed into crystalline structures, nor indeed into visible forms of any kind. Structureless fibrin gels obtained by this method are usually transparent and non-retractile, whereas the normal crystalline gel is opalescent and very retractile. If loosened from the sides of the vessel in which it has formed it shrinks, squeezing out serum, and if broken with a rod each piece rounds off to form a separate structure. The structureless clot on the contrary is a soft jelly—when cut by a rod the pieces instead of separating flow together on contact.

In the formation of the normal clot two main problems are presented for solution, the phenomenon of crystallization and the phenomenon of gelatinization. In regard to the former it seems evident that both the thrombin and the fibrinogen are essential to the crystalline form of aggregation, and this consideration would imply that both substances enter into the structure of the needles. Agents other than thrombin that cause a precipitation of the fibrinogen particles give only amorphous

aggregates. In regard to the cause of the gelatinization I have taken the general view advocated long ago by Nägeli, and expressed in more general terms recently by Höber. As applied to the specific case of fibrin this conception may be stated as follows: Fibrin is a hydrophilic colloid. Its particles attract and bind the surrounding water phase in an especial degree compared with similar hydrophilic colloids such as fibrinogen. When the fibrin particles or aggregates are greatly dispersed this action results in giving the system a greater degree of viscosity. In a lesser degree of dispersion a soft gel is formed, but when massed into the coarse aggregates of the crystalline needles the surrounding water films are so firmly bound as to form a solid gel.

THE FIRST PHASE OF COAGULATION—FORMATION OF THROMBIN

The Rôle of Calcium.—Arthus and Pagès demonstrated the fundamentally important fact that calcium is necessary to the process of the clotting of blood. If calcium is removed, as by oxalating the blood, no clotting occurs. If the calcium is restored by adding a proper excess of calcium chloride clotting occurs promptly. Schmidt was not willing to admit this important fact. He tried to prove that the action of calcium is not specific, but practically all later work has served to demonstrate that he was in error. The rôle of calcium is specific and can not be taken by any of the other bases normally present in blood. The theories advanced by Arthus and Pagès [22] and by Pekelharing [7] to explain the mode of action of calcium need not be discussed, since the careful experiments of Hammarsten [14] demonstrated that the calcium is concerned only in the formation of thrombin. In the second stage of clotting, the reaction between thrombin and fibrinogen, calcium is not necessary, although in certain concentrations it may influence the reaction especially as regards its velocity. We have little or no knowledge of the way that calcium acts. There is a difference of opinion in the first place as to whether or not calcium ions can cause the activation of prothrombin to thrombin when acting alone. Some workers believe that the calcium is effective in causing this change only when it acts in cooperation with the thromboplastic material of

the tissues (thrombokinase, cephalin). This question will be discussed below in connection with a description of the action of tissue extracts. It has been noted by many observers that the influence of calcium varies with its concentration. There is a certain optimal concentration at which the formation of thrombin is most favored, and beyond this point a further increase in concentration tends to inhibit or even prevent coagulation. Sabbatani,[23] believed that there is a certain minimal concentration below which the calcium ions are ineffective and also a maximal concentration that suffices to inhibit the activation. The maximal concentration he placed at 18 grms. calcium chloride per liter (molecular concentration 0.162). In this amount coagulation is prevented. Stassano and Daumas [24] state that the minimal concentration lies between 13 and 21 mgms. of calcium chloride per liter. The action of calcium may be prevented by precipitating it out of the blood in insoluble form, as an oxalate, for example, or by forming salts of calcium in the blood which have a small dissociation, so that the concentration in calcium ions is below the minimal limit referred to above. Sabbatani explains in this way the effect of citrates in preventing coagulation. Making use of fibrinogen solution with only traces of calcium Hammarsten was able to obtain specimens of fibrin in which the calcium content was only 0.007 per cent. If this calcium were contained in the fibrin as a constituent of its molecule it would imply a molecular weight too large (800000) to be accepted as possible. He concluded therefore that the calcium found was present as an impurity, and that it does not form a constituent of the fibrin, nor probably of the thrombin. Presumably from this point of view the calcium ions act as catalytic agents and not by forming a calcium compound with the prothrombin or any portion of its molecule. While this conclusion may be accepted provisionally it may be noted that it is scarcely a necessary result of the experiments reported by Hammarsten. If the fibrin needles consist of an orderly aggregation of fibrinogen and thrombin particles, the latter existing in small proportions compared with the former, it is possible that the calcium might be an essential constituent of the thrombin molecule without making a

percentage of the fibrin larger than the minimum obtained by Hammarsten. It would seem desirable to make an effort to determine whether an effective thrombin can be obtained entirely free from calcium.

The Rôle of Tissue Extracts.—The plasma of the circulating blood contains fibrinogen, prothrombin and calcium ions, but it does not clot, owing to the absence of active thrombin. If it were possible to get this plasma out of the body without coming into contact with the tissues, and without injury or destruction of any of the formed elements of the blood, then, no doubt, the plasma would remain fluid as it exists within the vessels. This experiment can be made in fact when one uses the blood of the lower vertebrates (bird, terrapin). By bleeding through a paraffined canula into a cooled paraffined centrifugal tube and centrifugalizing at once a plasma may be obtained which clots very slowly or not at all. A similar result might be obtained with the slowly clotting blood of the horse and possibly with other mammalian blood if sufficient care were taken. The prompt clotting that we observe ordinarily in shed blood is due to something added to the plasma when the blood escapes from its normal environment, and this something is derived either from the breaking down of some of the corpuscular elements of the blood itself or from the outside tissue with which it comes into contact. This fact has been demonstrated in various ways by many observers. In the mammals the platelets of the blood disintegrate very rapidly when the blood is shed and thus furnish one source for the substance that initiates the process of clotting. In the blood of the bird and the reptile there are no corpuscles as fragile as the mammalian blood-platelets, so that in these animals the initiation of the clotting depends mainly on material furnished by the outside tissue-elements, the injured tissue, for example, of the wound. Schmidt designated the material furnished by the blood corpuscles or the other tissue elements as zymoplastic substance, on the theory that it acts on the mother substance of thrombin, the prothrombin, and splits off active thrombin by a process of cleavage. Various other names have been given to this same material. Wooldridge [25] spoke of it as tissue-fibrinogen, Mora-

witz[26] as a thrombokinase, Fuld[27] and later Bordet and Delange[28] as a cytozym. Nolf included it under a general designation of thromboplastic substances, and frequently it has been referred to simply as tissue-extract. In my earlier papers I suggested the term thromboplastin on the view that the material is a definite chemical substance of unknown composition, but later when its chemical nature was revealed this term was abandoned.

Nature of the Zymoplastic Substance.—Information in regard to the chemical nature of this material has accumulated slowly, but its general trend has all been in one direction. Schmidt found that it is soluble in alcohol and ether, withstands boiling and on analysis shows the presence of phosphorus and nitrogen. He concluded, therefore, that lecithin must be present in the tissue extracts and must have some relation to their activity. Wooldridge also believed that the activity of his tissue-fibrinogen was referable to the lecithin contained in it, but he made the significant observation that while lecithin prepared from various animal tissues such as the brain, testis and thymus, is quite active, that obtained from the egg yolk is without effect on coagulation. Bordet and Delange state that their cytozym is thermostable and exhibits the solubilities of lecithin, and Hirschfeld and Klinger[29] corroborate this statement. Fonio in his advertised description of his patented preparation, so-called Coagulen-Ciba, which is a zymoplastic substance said to be obtained from blood platelets, speaks of the material as a lipoid. Zak[30] demonstrated that phosphatid material prepared from the brain (acetone precipitation of a petroleum ether extract of dried brain) has a strong zymoplastic action. It will be seen from these references that there has been a quite general agreement that the zymoplastic material is a lipoid substance and that it belongs to that group of lipoids known as phosphatids. My own work has led me to a similar conclusion.[31] I found that the active material may be extracted from dried tissues with ether, and that when the various constituents present in this extract are examined separately for their zymoplastic action the result shows that the active substance is a phosphatid soluble with difficulty in alcohol

and corresponding in its reactions with the phosphatid designated by Thudichum as cephalin. I was able to show that lecithin prepared from various sources has no zymoplastic action, while the related cephalin, from whatever tissue it is obtained, is, when freshly prepared, very active. The cephalin obtained from ether extracts of brain and other tissues withstands boiling without losing its zymoplastic activity. On the other hand it was found that aqueous or saline extracts of fresh organs which exhibit strong zymoplastic action are precipitated even at temperatures of 56° to 60° C. and lose their activity. If, however, the precipitate is collected, dried and extracted with ether a material is obtained which is zymoplastic. My interpretation of these results was that the active substance in tissue extracts is probably cephalin, but in the normal tissues the cephalin is associated with a protein having a very low temperature of heat coagulation, and very readily soluble in water. In this form the active material is thermolabile, since it is precipitated out of solution on heating, but the essential constituent, the cephalin, when separated off, as occurs in ether extracts, is not destroyed even at boiling temperature. In this way we may reconcile the opposing statements found in the literature of the subject in regard to the thermostability of the zymoplastic material. While the evidence collected pointed distinctly to cephalin as the essential zymoplastic substance there remained a possibility that the active substance might be an impurity of some kind adherent to the cephalin. At my request McLean undertook a special study of this point.[32] Cephalin was prepared with especial care from brain, liver and heart and in as pure a form as our present knowledge of this substance permitted. The material thus prepared exhibited always marked zymoplastic activity, while preparations of related phosphatids, lecithin, cuorin, heparphosphatid and sphingomyelin were inactive. It is difficult to say with certainty what peculiarity in the structure of the cephalin molecule confers upon it this especial property, but the evidence obtained seems to connect this particular activity with the unsaturated fatty acid group. McLean found that a hydrogenated cephalin furnished by Levene and West was inactive. Cephalin when freshly prepared is

usually very zymoplastic in aqueous solutions of 0.1 per cent or more. But the material, even though kept in the dark and in a desiccator loses its activity either slowly (through months) or rapidly (in weeks) according to its mode of preparation. An investigation of this point by McLean, soon to be published, indicates that the loss of activity is due to a process of oxidation and that parallel to this disappearance of its zymoplastic action there is a corresponding diminution in its iodine number.

The Mode of Action of the Cephalin.—The addition of zymoplastic material to the blood, from whatever source it is derived, initiates the process of clotting by leading to the development of active thrombin from the inactive prothrombin. How does it effect this result? The nature of the answers that have been given to this question may be illustrated by a brief statement of some of the theories proposed. Schmidt believed that the zymoplastic material acts on the mother substance of thrombin, the prothrombin, after the manner of an enzyme. The prothrombin undergoes cleavage with the formation of thrombin. It will be remembered that Schmidt did not accept the view that calcium salts are necessary to the formation of thrombin, and it is implied in his theory that the zymoplastic material may split off thrombin from prothrombin in the absence of calcium. This, however, is not the case. In a decalcified (oxalated) plasma no thrombin is formed by the addition of calcium free zymoplastic material (Cephalin). This important fact indicates that the zymoplastic material and the calcium cooperate in the production of thrombin from prothrombin, and most of the later views are founded on this assumption.

Fuld [33] and Morawitz [34] were influenced in their theories by the recently discovered action of enterokinase in activating trypsinogen to trypsin. They suggested that tissue extracts contain an organic activator or kinase which Morawitz designated as thrombokinase, and that an essential condition for its activity is the presence of calcium ions. In other words, zymoplastic material is a kinase which in co-operation with calcium activates prothrombin to thrombin. Bordet and Delange [28] have accepted this view in its essential features, but they have introduced an

additional factor beside adopting a different nomenclature. They believe that the zymoplastic material, designated by them as cytozym, in the presence of and presumably with the co-activity of calcium reacts with a substance designated as serozym to produce thrombin. Their serozym corresponds with the prothrombin (thrombogen) of Morawitz, but they assume further that the serozym exists in the circulating blood in an antecedent stage, a proserozym. At the time of clotting the proserozym is converted to serozym by some unknown reaction, possibly the removal of an antagonistic substance. According to their view this latter reaction is the initial step in the process of clotting, but so far as I know, they give no theory or fact in regard to the cause of this change. Morawitz in his numerous publications has expressed no definite opinion in regard to the nature of his thrombokinase. The typical kinase, the enterokinase of the small intestines is supposed generally to be an enzyme, but if we accept the results of the work described above thrombokinase is a definite chemical substance, cephalin, whose action can scarcely be interpreted as that of an enzyme. Detailed study of its action indicates that a given amount of cephalin occasions the conversion of a definite amount of prothrombin to thrombin (Gasser). There is no evidence of an enzyme characteristic in this reaction, so that the nomenclature of thrombokinase or cytozym as applied to cephalin would seem to be inappropriate. In common with other workers I am convinced that the conversion of prothrombin to thrombin requires, under the conditions existing in the circulating blood, the combined action of the calcium ions and the cephalin, but I have attempted to explain the action of the latter from a different point of view. Instead of assuming that the cephalin acts upon the prothrombin as a kinase or enzyme or in any other way I have adopted the other possible suggestion, namely, that under normal conditions the prothrombin is protected from the activating influence of the calcium ions by a combination of some kind with an inhibitory agent or antisubstance, and that the cephalin exerts its accelerating effect upon coagulation by neutralizing the influence of this inhibitory substance thus liberating the prothrombin so that the calcium can

convert it to thrombin. The basis for this point of view is found in the fact that there exists in the blood a substance which prevents or tends to prevent coagulation. This substance has been referred to by many observers, usually under the name of antithrombin. I have described a simple method, referred to below, by means of which a relative estimate may be made of the amount of this substance present. Observations by this method have shown that in the slow coagulating bloods, such as those of the lower vertebrates or the blood of a peptonized dog, the amount of antithrombin is increased and that the greater the amount of this antithrombin the more stable or less coagulable is the blood. Tissue extracts or cephalin solutions added to such bloods cause a shortened coagulation time or induce coagulation in those specimens in which spontaneous coagulability is no longer exhibited. It can be demonstrated moreover that the addition of cephalin to such plasmas causes a more or less complete disappearance of the detectible antithrombin. This fact may be illustrated by the following experiments made upon the plasma from oxalated blood.

The blood of the animal was oxalated as it flowed from the artery. The mixture was centrifugalized and the clear plasma pipetted off. With this plasma two mixtures were made: Mixture A, consisting of plasma one part and water one part—and Mixture B, consisting of plasma one part and an aqueous solution of cephalin one part. The mixtures were allowed to stand for 30 minutes and were then heated to 54° C. to precipitate the fibrinogen. The filtrates from these precipitates were then tested for antithrombin according to the following schema:

I. Mixture A, 1 drop + Thrombin 5 drops — Incubation of 15 mins. + Fibrinogen 10 drops = Clot in 5 mins.

Mixture A, 1 drop + Thrombin 4 drops — Incubation of 15 mins. + Fibrinogen 10 drops = Clot in 10 mins.

Mixture A, 1 drop + Thrombin 3 drops — Incubation of 15 mins. + Fibrinogen 10 drops = Clot in 15 mins.

Mixture A, 1 drop + Thrombin 2 drops — Incubation of 15 mins. + Fibrinogen 10 drops = Membrane-clot in 20 mins.

In the similar series made with Mixture B all of the tubes clotted within 3 minutes showing that the antithrombic action of the plasma had been completely removed by the cephalin.

A more striking result may be obtained by using pig's plasma which contains a larger amount of antithrombin. For example—with a freshly oxalated specimen of pig's blood some clear plasma was obtained and two mixtures A and B were made as above, with water and with an aqueous solution of cephalin. After standing for 30 minutes these mixtures were heated to 54° to remove the fibrinogen and the filtrates were tested for antithrombin with the following results:

I. Mixture A, 1 drop + Thrombin 5 drops — Incubation of 15 mins. + Fibrinogen 10 drops = Partial clot in 65 mins.

Mixture A, 1 drop + Thrombin 4 drops — Incubation of 15 mins. + Fibrinogen 10 drops = No clot in 2 hrs.

Mixture A, 1 drop + Thrombin 3 drops — Incubation of 15 mins. + Fibrinogen 10 drops = No clot in 2 hrs.

Mixture A, 1 drop + Thrombin 2 drops — Incubation of 15 mins. + Fibrinogen 10 drops = No clot in 2 hrs.

II. Mixture B, 1 drop + Thrombin 5 drops — Incubation of 15 mins. + Fibrinogen 10 drops = Clot in 5 to 10 mins.

Mixture B, 1 drop + Thrombin 4 drops — Incubation of 15 mins. + Fibrinogen 10 drops = Clot in 5 to 10 mins.

Mixture B, 1 drop + Thrombin 3 drops — Incubation of 15 mins. + Fibrinogen 10 drops = Clot in 5 to 10 mins.

Mixture B, 1 drop + Thrombin 2 drops — Incubation of 15 mins. + Fibrinogen 10 drops = Clot in 10 to 15 mins.

When blood-serum is used instead of blood-plasma a different result is obtained; addition of cephalin solutions causes little or no diminution in the amount of free antithrombin. The difference in the behavior of the antithrombin in plasma and in serum is difficult to understand. It depends apparently upon the fact that in serum fully formed thrombin is present; and, as will be described further on in discussing metathrombin, thrombin and antithrombin tend to combine to form first a loose and finally

a firm compound, and upon this combination the cephalin has apparently little effect.

These considerations led me to believe that in the circulating blood antithrombin protects the prothrombin by some means and that the efficacy of the cephalin resides in its property of neutralizing the antithrombin. The evidence that I have been able to present in favor of this view has been indirect only, namely, the existence of antithrombin in the blood and the power of the cephalin to neutralize this antithrombin. Some recent work in my laboratory has brought to light the additional fact that in some of the tissues, liver, heart-muscle, lymph glands, there is a phosphatid material which has a quite specific power of acting upon or combining with prothrombin so as to prevent its activation to thrombin. Whether or not this new substance, which provisionally I have designated as antiprothrombin, will be found as a normal constituent of the blood can not be stated at present. It seems very possible that this may be the case, and in that event it will be necessary to consider its especial function. It may be that the inhibiting substance which according to my view protects the prothrombin from activation and thus safeguards the fluidity of the blood may be this antiprothrombin rather than the antithrombin. Experiments that are described farther on show that cephalin is capable of liberating prothrombin from the inhibitory influence of the antiprothrombin and therefore has the property of neutralizing the action of both of the inhibitory substances, the antiprothrombin and the antithrombin. Further experiments must be made to determine definitely whether the action of cephalin in clotting is to be explained along these lines or, according to the hypothesis of Morawitz, as the result of some direct action exerted by it upon the prothrombin.

Cephalin as a Hemostatic.—On any theory of their action tissue-extracts are of the first importance in starting and accelerating the clotting of blood, and one might suppose therefore that such extracts could be used to control hemorrhages. This thought has occurred no doubt to many workers and some of the attempts to prepare the zymoplastic substance in a form suitable for such uses have been put on record, for example, the prepa-

ration of thrombokinase described by Horneffer and Battelli [35] and the patented preparation of Kocher and Fonio placed on the market for this purpose. With regard to the latter it may be observed that it professes to be a preparation of lipoid material obtained from blood-platelets. Since the same lipoid material exists in much greater abundance in tissues like the brain which are much more accessible it is difficult to understand the reason or value in selecting platelets as a source of supply. According to my views the active constituent of zymoplastic substance is cephalin and since this material may be prepared very readily in active form, the question arises whether this cephalin may be put to some practical use in controlling hemorrhage. It is a question which I am having investigated with care as I believe it promises useful results. Meanwhile I should like to put on record some incomplete results obtained by myself and others that give some indication of the lines to be followed. Impure but very active preparations of cephalin may be made by the method used in my laboratory as follows:

Preparation of Cephalin.—Fresh brains are obtained from the slaughter house, cleaned from membranes and blood, ground to a pulp, spread thin upon glass plates and dried in a current of warm air. When thoroughly dry, powder in a mortar and then extract with ether for 3 or 4 hours. Filter off the ether in a closed space and repeat the filtration until the filtrate is clear. Evaporate the filtrate to dryness before an electric fan. Extract the residue thoroughly with acetone (to remove cholesterol and cholesterol esters). Drain off the acetone and extract twice with excess of alcohol (to remove most of the lecithin). Drain off the alcohol, allow to dry and keep in a desiccator in the dark. For use a small portion of the material is stirred in water until a milky solution is obtained—a 0.1 per cent. solution is convenient for use. If necessary it may be sterilized by boiling.

Whether or not these solutions can be used advantageously in controlling hemorrhage must be determined by experience. Two general facts, however, must be borne in mind. In surgical operations in which there may be much laceration of tissue there is probably a liberation of much zymoplastic material, and addition of cephalin solutions may therefore be superfluous. Secondly, cephalin in solid form or in solution deteriorates, slowly

or quickly according to circumstances, and every preparation should therefore be tested before using. One relatively simple method of testing the cephalin which is employed in my laboratory makes use of its great activating influence on fresh serum. An animal is bled—part of the blood is oxalated and centrifugalized to obtain oxalated plasma, and a part is clotted and centrifugalized to obtain serum. In dog's or cat's serum the amount of effective thrombin is not large, so that if say 3 drops of the serum are added to 8 drops of the plasma clotting of the latter takes place slowly and often imperfectly, requiring 30 minutes or more. If, however, one adds to the 3 drops of serum 3 or 4 drops of the cephalin solution and then adds the plasma the latter will clot firmly as a rule in one minute or less.

Making use of aqueous solutions of cephalin I have been able to control obstinate hemorrhages in hemophilic cases by applying a dressing of gauze soaked in the solution, although prompter and more satisfactory results were obtained by using both thrombin, in powder, and the cephalin. Cecil has described [36] a special method of using cephalin to control hemorrhage after prostatectomy with which good results have been obtained, and no doubt under proper conditions this material may be employed to advantage in controlling external hemorrhage. I have been interested also in another possibility, namely, the control of internal hemorrhages in cases with a more or less marked hemophilic tendency. It would seem possible that cephalin introduced into the blood directly or by absorption from the intestines might be used safely to lower its coagulation time. With this idea in mind I have made a large number of experiments on dogs in which cephalin in aqueous solution was injected intravenously, intraperitoneally or subcutaneously or finally was fed by mouth. The results of these experiments have not been published since they showed many irregularities that will require further experimentation to explain. Some general results, however, may be referred to briefly. Intravascular injection of cephalin in dogs in the proportion of one decigram per kilogram of animal causes a shortening of the coagulation time of the blood by $\frac{1}{3}$ or $\frac{1}{2}$ of the normal time, and this effect may endure for at least one

or two hours without any evidence of intravascular clotting and no evidence of a general reaction upon the animal, so far as respiration, heart-rate or blood pressure is concerned. When cephalin is added to blood outside the body it brings about a rapid production of thrombin. It is to be presumed that when it is introduced into the circulating blood there is also a production of thrombin, but there is no intravascular clotting. The explanation that I would suggest is that this free thrombin is bound by the antithrombin, especially as it has been shown [44] that at the body temperature this property of the antithrombin is much augmented. Gasser [37] has made it very probable that the antithrombin plays this rôle of safeguarding the blood from the action of free thrombin. This method of using cephalin needs more careful study since the reaction differs somewhat in different animals and the nature of the after-effects have not been determined.

Very suggestive results were obtained by giving the cephalin by mouth. Evidence was obtained that in this way enough may be absorbed to have a distinct and prolonged effect in lowering the coagulation time of the blood. As an example, the following experiment may be quoted: A dog weighing 6 + kilograms was placed under morphia. Specimens of blood were taken from the jugular vein for the normal coagulation time. 200 c.c. of water were then given by stomach tube and observations were made on the coagulation time during the subsequent two hours. The stomach was then emptied and 200 c.c. of a 0.5 per cent. aqueous solution of cephalin were introduced, and observations on the coagulation time were made during the next four hours. The following results were obtained. The coagulation times were determined in duplicate.

1. Normal specimen Before injection.	Coagulation time of 2 cc.	a = 34 to 35 mins. b = 35 mins.
	Gave 200 cc. water by stomach tube	
2. Specimen 1 hr. After water.	Coagulation time of 2 cc.	a = 36 mins. b = 40 mins.
3. Specimen 1 hr. 40 mins. After water,	Coagulation time of 2 cc.	a = 34 to 35 mins. b = 36 to 38 mins.

Stomach emptied (recovered 85 cc.) and then filled with 200 cc. of the solution of cephalin

4. Specimen 40 mins. After Cephalin	Coagulation time of 2 cc.	a = 30 mins. b = 32 mins.
5. Specimen 1 hr. 40 mins. After Cephalin	Coagulation time of 2 cc.	a = 25 mins. b = 26 mins.
6. Specimen 3 hrs. After Cephalin	Coagulation time of 2 cc.	a = 28 mins. b = 28 mins.
7. Specimen 4 hrs. After Cephalin	Coagulation time of 2 cc.	a = 23 mins. b = 24 mins.

On the strength of the results obtained from such experiments I have given cephalin by mouth to hemophilic cases to arrest hemorrhage. The results have seemed to be favorable. For example, one of the boys with congenital hemophilia whose cases I have described previously [38] reported with a bleeding tooth. The tooth was badly decayed so that the crown broke off and obstinate bleeding set in. Efforts to control this bleeding by local applications of thrombin and cephalin were not entirely successful owing to the difficulty of keeping the packing in position. He was given 100 cc. of cephalin solution twice daily, before breakfast and at bed time. The bleeding stopped promptly and no further difficulty was experienced. I have had occasion to treat in the same way a number of persons suffering from hemophilic joints. Unfortunately most of these cases were at a distance and observations on the blood were not possible, except in two instances. One of the latter was a boy of 10 years admitted to the Harriet Lane Home with a history of joint troubles and long continued bleeding from slight injuries. Examination of his blood showed a hemophilic condition, although the family history gave no indication of a hereditary factor. The coagulation time and the prothrombin time were taken. By prothrombin time is meant the time of clotting of the oxalated plasma when recalcified with an optimum amount of calcium. In normal human beings with the procedure I use it is equal to 10 mins. plus or minus. The boy was given 100 cc. of a solution of cephalin daily for 3 weeks while in the hospital, and subsequently the same amount daily on alternate weeks over a period of 6 months. The blood examinations were as follows:

Before treatment	Coagulation time 2 cc.	= 75 to 80 mins.
	Prothrombin time	= 55 to 60 mins.
After 3 weeks' daily treatment..	Coagulation time 2 cc.	= 80 mins.
	Prothrombin time	= 43 mins.
After six months' treatment alternate weeks	Coagulation time 2 cc.	= 50 mins.
	Prothrombin time	= 28 mins.

The boy 's condition had improved and although the examinations were fewer than desirable they seemed to show that the cephalin had influenced the condition of the blood in the right direction.

A second case was treated in a similar way under the direction of Dr. C. R. Drinker who kindly gave me a complete history together with the results of the blood examinations. The patient, a young man 20 years of age, entered the Peter Bent Brigham Hospital with a long history of swollen and painful joints and frequent severe and dangerous hemorrhages. He was given cephalin solution by mouth daily over a period of 13 weeks. Four examinations of the blood were made.

Before treatment..January 22....	Coagulation time	= 47 minutes
	Prothrombin time	= 32 minutes
March 23...	Coagulation time	= 50 minutes
	Prothrombin time	= 28 minutes
After 7 weeks' treatment	Coagulation time	= 35 minutes
	Prothrombin time	= 23 minutes
After 9 weeks 'treatment	Coagulation time	= 30 minutes
	Prothrombin time	= 24 minutes
After 13 weeks' treatment	Coagulation time	= 34 minutes
	Prothrombin time	= 19 minutes

The patient reported a marked improvement in his condition.

These incomplete observations need further confirmation—they are reported simply as an indication that the coagulation time in hemophilic bloods can be moved toward the normal by the ingestion of solutions of cephalin. The treatment is simple and not attended by any untoward symptoms and the results obtained so far are sufficiently encouraging to warrant a careful trial of the method under conditions more favorable for observation.

The Means for Retarding or Preventing the Coagulation of Blood.—There are many methods known by which the coagulation

of the blood may be retarded or prevented; for example, by cooling, by the addition of neutral salts to a certain concentration, by the precipitation of the calcium of the blood, etc. Under certain pathological conditions such as phosphorus or chloroform poisoning or hepatic cirrhosis, which are associated with liver injury or insufficiency, there may occur a marked diminution in the fibrinogen content of the blood and a consequent retardation and imperfection in the clotting, as has been shown by Whipple and Hurwitz [39] and Whipple.[40] But among the factors of this kind which occur normally in the body and are subject to possible variations under pathological conditions the most interesting at present are the so-called antithrombin and a second substance, lately brought to light, which for want of a better name may be designated as antiprothrombin. I should like to call your especial attention to these two substances.

Antithrombin.—Nearly all of the important contributors to the literature of coagulation have referred to the presence in the blood of an inhibiting factor, or have at least considered the possibility of the existence of such a factor. Schmidt [1] in the final summing up of his views on coagulation suggests the idea that there may be a something normally present in the blood that hinders the action of thrombin, a substance therefore which might be designated as antithrombin. In his book he devotes much space to a description of the anticoagulating action of two proteins or conjugated proteins, cytoglobin and preglobulin, which he obtained from many tissues. He believed that these proteins contain an inhibiting group or radicle which possibly may be split off and be found in the blood, but his further description of the action of these substances indicates that they would fall into the group of the antiprothrombins rather than the antithrombins. Nolf in his theory of coagulation [41] recognizes the existence in the blood of an antithrombin or, as he prefers to call it, an antithrombosin, and he attributes to it an important part in the maintenance of the fluidity of the blood.

In Nolf's theory thrombin does not assume the importance attributed to it in other theories. Like fibrin it is the product of a reaction of thrombogen, thrombozyn and fibrinogen. It con-

stitutes in fact an intermediate or imperfect form of fibrin. Consequently the action of the antithrombin is interpreted not as preventing the action of thrombin on fibrinogen but as retarding or preventing the reaction of the three factors just mentioned. Bordet and Delange [28] in speaking of the conversion of the pro-serozym to serozym refer in a guarded way to the possible removal of an antagonistic substance normally present in blood. Morawitz recognizes the existence of a substance or substances in plasma and serum which inhibit coagulation and considers it probable that they play a rôle in the circulating blood—although in his theory of coagulation this rôle of an antithrombin is not taken into account. In peptone blood and in avian blood the existence of an antithrombin is accepted by many authors. Schickele [42] asserts that in the tissue juice obtained under pressure (press-saft) from a number of tissues, particularly from the uterine mucous membrane, there is contained an antithrombin. In a long series of papers Doyon [43] with a number of co-workers has described several methods by which an antithrombin may be obtained from various organs. In their later papers the claim is made [44] that this antithrombin is a nucleic acid or nucleic acid complex. They state that nucleic acids of animal or vegetable origin prevent the coagulation of blood in vitro.

In looking over the literature bearing upon this point it will be noticed that one difficulty and cause of confusion is that few, if any, authors, except possibly Schmidt, make a distinction between antithrombin proper, that is to say a substance that prevents or retards the action of thrombin on fibrinogen, and inhibiting substances that prevent the formation of thrombin by combining with or neutralizing in some way the prothrombin.

The importance of this distinction is shown in the next section. Throughout my work on this subject I have used the term antithrombin to designate a substance that prevents the action of thrombin on fibrinogen, in the way illustrated so markedly by hirudin which is the typical antithrombin. I have devised a simple method of detecting antithrombin and of expressing with some accuracy the relative amount present. The method depends upon the use of purified thrombin and fibrinogen prepared

according to the methods described at the beginning of this paper; it is carried out as follows: In a series of four tubes (homeopathic vials 75 by 15 mm.) one places respectively 2, 3, 4 and 5 drops of a suitable solution of throbbin—one then adds to each tube 10 drops of a fibrinogen solution and notes the time of coagulation. This series constitutes a control, and in my experiments the strength of the thrombin solution was such as to cause coagulation in all the tubes within five minutes. For the liquid supposed to contain antithrombin one makes a similar series of four tubes containing respectively, 2, 3, 4 and 5 drops of the thrombin solution. To each of these tubes one adds a single drop of the liquid under investigation, allows a certain definite period of incubation (I have used generally 15 minutes) and then adds 10 drops of fibrinogen to each tube—the time of coagulation is noted and the delay over the control will be in proportion to the antithrombin present. It is quite important in making this test to give a certain period of time for the combination or action, whatever it may be, of the antithrombin and thrombin. The power of the antithrombin to combine or neutralize thrombin is a function of the time, as is illustrated by the accompanying figure. When the solution investigated contains small amounts of antithrombin this fact may be missed if the fibrinogen is added at once to a mixture of the thrombin and antithrombin. This factor of time in the reaction between these two substances has been noted by a number of observers who have dealt with the power of serum or plasma to neutralize free thrombin added to it.*

* This precaution has been entirely overlooked in some recent work published by Dale and Walpole (The Biochemical Journal, 1916, 10, 331). These observers prepared a solution of prothrombin which they examined for the presence of antithrombin. They found none—whether or not their result is correct I cannot venture to say until I have had an opportunity to repeat their work. But it may be said positively that the method they adopted to detect antithrombin was not adapted to reveal its presence, unless there was a large quantity. On the contrary their method would have destroyed any if it had been present. They neglected in the first place to give a period of incubation before adding the thrombin, and in the second place they heated the solution of prothrombin to 60° C. for twenty minutes, a procedure which would have weakened greatly or destroyed the antithrombin. In the third place they did not purify their thrombin, but used an impure preparation containing tissue extract (Kinase).

Using this method I have been able to show the presence of an antithrombin in the plasma and the serum of blood of a number of mammals (man, dog, cat, rabbit, pig). The amount of antithrombin present varies somewhat in the animals of any one species, and to a more marked extent in the animals of different species. As a rule the cat's blood contains the least antithrombin among the animals examined; next to the cat comes man, and then

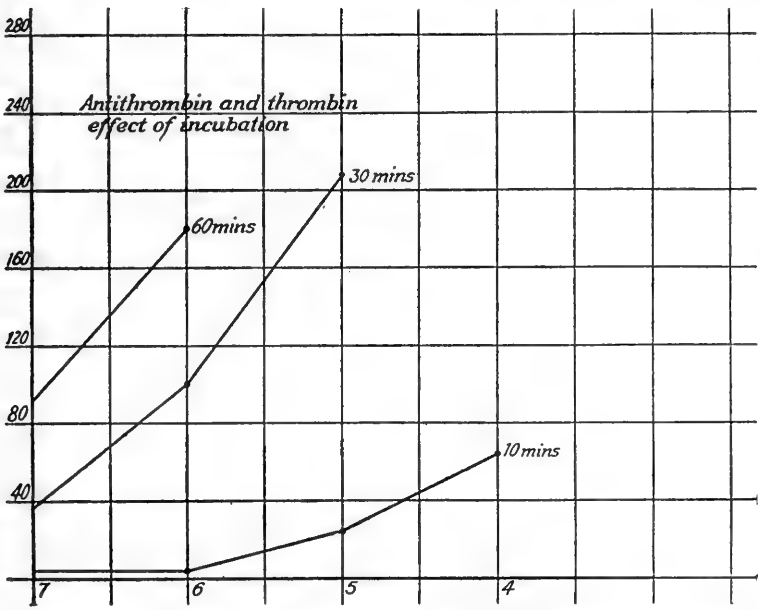

FIG. 2.—To illustrate the effect of the period of incubation of a mixture of thrombin and antithrombin upon the neutralizing action of the latter. The figures on the abscissa indicate the number of drops of a thrombin solution to which was added one drop of the antithrombin solution. The ordinates indicate the time in minutes necessary for coagulation when fibrinogen was added to the several mixtures after periods of incubation of ten, thirty and sixty minutes. The ordinates represent therefore the extent of the antithrombic action.

in series, the dog, rabbit and pig. The usual relation of the antithrombin in the blood of man, dog and rabbit is illustrated by the accompanying figure.

Minot and Denny [45] have attempted to study the antithrombin quantitatively by my method in various pathological conditions in man. In each determination it was necessary to take the blood

of a normal man as a standard for comparison. Their results are expressed in terms of what they call the antithrombin factor. This factor was obtained from the tubes in which the clotting occurred within a convenient period of time (8 to 25 minutes). The sum of the added times for the several tubes of the control was divided into the added times for the corresponding tubes of the case examined. The normal individuals showed some varia-

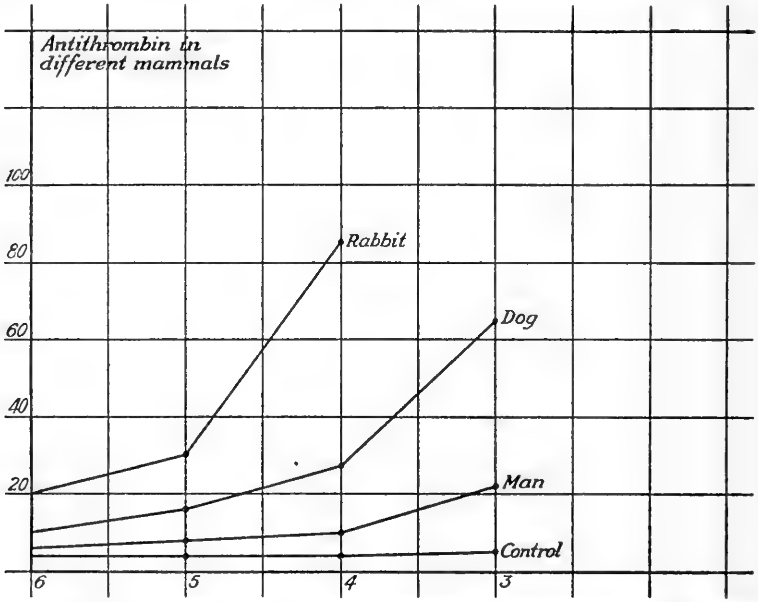

FIG. 3.—To illustrate the relative amounts of antithrombin in the blood plasma of man, dog and rabbit. The figures along the abscissa indicate drops of thrombin solution to which were added one drop of the oxalated plasma, after heating to remove fibrinogen. The ordinates represent the time of clotting in minutes after addition of ten drops of fibrinogen solution to each mixture. In each case the period of incubation of the thrombin and antithrombin solutions was fifteen minutes.

tions, the limits of which were expressed in a similar factor by dividing the added times of the case with the highest antithrombin by that of the lowest antithrombin content and the reverse.

Expressed in this way the normals ranged from 0.82 to 1.21. Among the pathological cases studied by them some had a distinct increase in antithrombin, *e.g.,* hemophilia with a factor of 2.3

to 2.4 and acute splenomyelogeneous leucemia 1.9 to 2.4, while others showed a subnormal amount, *e.g.*, thrombosis, 0.55 and jaundice, cirrhosis, 0.55. In the blood of the bird and the reptile (terrapin) the amount of antithrombin is much larger than in the mammal, and this fact may be assumed to furnish the explanation of the longer clotting time of these bloods when obtained from the vessels without contact with tissue juice. In the blood of dogs that have been peptonized successfully so as to obtain an incoagulable blood the amount of antithrombin is increased very greatly. This fact may be illustrated by the figures of one of my earliest experiments. The specimens of blood were oxalated, centrifugalized and the clear plasma was heated to 60° C. to remove the fibrinogen.

Antithrombin before peptonization:

1. Thrombin 5 drops + Heated plasma 1 drop — incubation of 10 minutes + Fibrinogen 10 drops, clotted in 10 mins.
2. Thrombin 4 drops + Heated plasma 1 drop + incubation of 10 mins. + Fibrinogen 10 drops, clotted in 15 mins.
3. Thrombin 3 drops + Heated plasma 1 drop + incubation of 10 mins. + Fibrinogen 10 drops, clotted in 25 mins.
4. Thrombin 2 drops + Heated plasma 1 drop + incubation of 10 mins. + Fibrinogen 10 drops, clotted in 1 hr. 45 mins.

Antithrombin 30 *mins. after peptonization:*

1. Thrombin 5 drops + Heated plasma 1 drop + incubation of 10 mins. + Fibrinogen 10 drops. No clot in 24 hrs.
2. Thrombin 4 drops + Heated plasma 1 drop + incubation of 10 mins. + Fibrinogen 10 drops. No clot in 24 hrs.

With 3 and 2 drops of thrombin the same result was obtained.

A reëxamination of the blood of the dog after 24 hours showed that its antithrombin content had returned nearly to the normal level.

The Nature of Antithrombin—Its Thermolability.—Nothing is known definitely concerning the nature of the antithrombin of blood. The active principle known as hirudin secreted by the salivary gland of the leech is a very strong antithrombin, more powerful apparently than the substance found in blood. According to the observations of Franz [46] hirudin is a protein belonging to the proteose group. It has a high degree of thermostability. Heating to 80° C. has no effect on its activity; heating to 100° C.

while it weakens its action does not destroy it completely. According to some authors (Dickinson) prolonged boiling does not destroy the activity of leech extracts.

I have made a number of efforts to separate antithrombin from the blood, but so far have not been successful. After precipitating blood plasma with acetone, for example, I have not been able to find antithrombin in either the precipitate or the filtrate. Apparently it is destroyed by the acetone.

As stated above, Doyon and his co-workers have obtained a substance from the tissues which they designated as antithrombin. His earlier experiments indicated that he was dealing with a stable substance which withstands boiling, precipitation by strong acid, etc. In his later experiments he identifies his antithrombin as a nucleic acid or a compound of nucleic acid, but, as stated in the next section, there is good reason to believe that the material he was studying was not an antithrombin proper.

The antithrombin of blood is distinctly thermolabile. I have not made a special study of the degree of its thermolability in different bloods. In this respect the time factor is a matter of great importance. Strong peptone plasmas heated to 80° to 85° C. lose their antithrombin activity completely. According to Gasser [37] the antithrombin in mammalian plasma is nearly completely destroyed by heating for five minutes to 65° C. In my own experiments I have found that it is destroyed at 70° C., and that heating to 63°–°64° C. for five minutes in some cases also practically removes this action of plasma. In fact heating to only 60° C. for a minute or less weakens distinctly the antithrombic action of plasma. In my earlier experiments all plasmas in testing for antithrombin were heated slowly to 60° C. to remove the fibrinogen and any traces of thrombin that might be present, but in my later experiments I have found it preferable to heat the plasma slowly to the temperature just sufficient to coagulate the fibrinogen. In oxalated plasmas this coagulation occurs at 53° to 54° C. and the plasmas so heated show a greater antithrombic action than if heated to 60° C. These observations all show that the antithrombin of mammalian blood is markedly thermolabile, and in this respect differs from solutions of pure

hirudin or leech extracts. It is possible that the thermolability of the mammalian antithrombin may be due to the condition in which it exists in the plasma, and that if isolated it would possess a greater degree of thermostability. But so far as our knowledge goes it is more probable that mammalian antithrombin is not identical in composition with the leech hirudin.

Source of the Antithrombin.—A number of authors—Delezenne,[47] Nolf,[48] Popielski[49]—have stated that the antithrombin in mammalian blood, particularly the antithrombin formed after the injection of peptone solutions is produced in the liver. This conclusion is corroborated by the experiments of Denny and Minot[50] in which the determinations of antithrombin were made by my method. They found that long stasis of blood in the liver, as well as perfusion of the organ, was accompanied by a distinct increase in the antithrombin content, and that similar results were not obtained from other organs. Destruction of liver tissue, as in phosphorus poisoning, occasions on the contrary a marked decrease in antithrombin. Outside the variations noted under pathological conditions, which have been referred to briefly above, changes in the antithrombin content of the blood may be induced by several experimental methods. Peptonization in the dog, as previously stated, causes a very marked increase in the antithrombin. A large literature has developed in connection with this action of peptone, and a number of different views have been held in regard to the substance in the peptone solution which is responsible for the reaction, and the way in which this substance acts. While most authors agree that the liver plays an important rôle the manner of its action has been variously interpreted. According to some the liver cells secrete the anticoagulating substance, while others assume that the endothelial cells are the responsible tissue. Delezenne attributes only an indirect influence to the liver cells in line with the theories of Lilienfeld.

It is not possible to arrive at any satisfactory conclusion upon the matter from a study of the literature alone. The subject needs further investigation, but one may feel confident at least that peptonization in the dog causes in some way a notable augmentation of the antithrombin in the blood. A second method of

increasing the antithrombin is by the intravascular injection of thrombin. Davis [51] found that intravascular injection of relatively large amounts of purified thrombin (28 to 56 milligrammes to each kilogramme of animal) instead of causing intravascular clotting as might have been expected brought about for a short period a prolongation of the clotting time. In later unpublished experiments he was able to show that there was an actual increase in antithrombin, although the reaction, as in the case of peptone injections, was subject to some irregularities. It would seem in this case as though the excess of thrombin had caused a protective secretion or formation of antithrombin.

A variation in antithrombin in the other direction, a decrease, may be produced, as I have shown repeatedly in laboratory experiments, by intravascular injections of solutions of cephalin or of tissue extracts, when used in sufficient quantity. A more interesting example of a similar change is described by Drs. K. R. and C. K. Drinker.[52] These authors found that rapid progressive hemorrhage causes a progressive shortening of coagulation time, and a parallel or greater diminution in antithrombin. The diminution in this case may be due to an actual decrease in production, but our knowledge of the normal rate of production and of the normal fate of the antithrombin is much too imperfect to warrant any positive conclusion of this kind.

We know that antithrombin when present in excess in the blood, *e.g.,* after peptonization, is removed rather promptly, and experiments show that its removal is not due to elimination through the kidneys, or, at least, not solely through the kidneys. The manner in which it is neutralized or removed has not been definitely discovered, although suggestive indications of a possible mechanism adapted to this purpose are furnished by recent observations upon the nature of metathrombin.

Metathrombin.—We owe the name of metathrombin to Morawitz, and a clear expression of the phenomenon which it indicates to him and to Fuld, although here again the initial observation goes back to the fruitful investigations of Schmidt. Metathrombin is the name given to an inactive or combined form of thrombin found in serum. It is said not to be present in plasma and its

formation is connected therefore with the process of clotting. Its presence in the serum is demonstrated by the fact of alkali or acid activation—the fact, namely, that the addition of alkali or of acid to a serum followed by subsequent neutralization, results in a marked increase of the active thrombin in the serum. The method suggested by Morawitz of carrying out this experiment is to add to the serum an equal volume of N/10 Na OH, and after standing for 15 minutes to neutralize with N/10 HCl. It is convenient in making this experiment to add a drop of solution of neutral red to the serum as an indicator in the process of neutralization. The origin of this metathrombin has been difficult to explain. It is absent, or at least not demonstrable in the plasma, and appears in the serum very quickly after clotting occurs. Fuld [53] believed that it is a modified or inactive form of thrombin and assumed that the latter substance in solution does not remain unaltered but tends to pass over into the more stable but inactive form of metathrombin. This simple explanation is not satisfactory, for it may be shown easily that aqueous solutions of purified thrombin are quite stable and in any event do not yield metathrombin no matter how long they are kept. It is not probable, therefore, that the metathrombin is formed by direct alteration of the thrombin. Still it seems evident, as Morawitz and Fuld have pointed out, that the thrombin formed in clotting passes over in some way into metathrombin, and the often observed disappearance of free thrombin from serum has been connected naturally with the formation of metathrombin. It has been noted, moreover, by several observers that when free thrombin is added to serum it disappears rather rapidly, and this capacity of the serum to destroy thrombin is remarkable when contrasted with the stability of thrombin in simple aqueous or saline solution. When the serum is heated to 65° to 70° C. its power in this respect is lost. These facts suggest the view that in serum or plasma there is contained some thermolabile substance which is responsible for the inactivation of thrombin, and it would seem probable that this substance inactivates the thrombin by combining with it, so that metathrombin is to be regarded not as an altered thrombin but as a combined thrombin. Wey-

mouth [54] in a careful study of the free and combined thrombin in sterile and non-sterile sera kept for long periods came to the conclusion that the inactivating substance in serum is antithrombin and that so-called metathrombin is a combination or compound of thrombin and antithrombin. His conclusion has been supported in a more recent paper by Gasser,[37] who furnishes a number of interesting observations upon metathrombin and some direct as well as indirect evidence in favor of the view that it is a combination of thrombin and antithrombin. He showed for example that purified thrombin added to oxalated plasma, which contained no metathrombin and which had been heated to 60° C. to remove its fibrinogen, yielded, after a suitable period of incubation, a solution that contained no free thrombin but distinct amounts of metathrombin. The argument is that the antithrombin in the heated plasma combined with the thrombin to form a metathrombin. In his experiments Gasser brings out clearly an important distinction in the manner of activation of serum by alkali treatment and by tissue extracts (kinase, cephalin). In fresh serum, as has been long recognized, both active and inactive thrombin are present, and the latter exists in two forms, first as the so-called prothrombin which by the addition of cephalin can be activated to thrombin by calcium salts, and second, the so-called metathrombin on which apparently tissue extracts (cephalin) have no effect, but which can be dissociated with the liberation of active thrombin by the action of alkali or acid. The amount of prothrombin left in the serum depends upon the quantity originally present in the plasma, and the proportion of this original amount that at the time of coagulation has been freed by the influence of the zymoplastic substance (cephalin). The greater the amount of this latter material set free in the blood, or added to it at the time of coagulation, the smaller will be the residuum of prothrombin that goes over into the serum. If the view of the nature of metathrombin described above is correct it should follow that conditions capable of liberating active thrombin in the circulating blood should occasion also the appearance of metathrombin in the plasma. This matter has been the object of a special study made in my laboratory

during the present year by Mr. A. R. Rich. He has added to the circulating blood, by intravascular injections, relatively large amounts of cephalin, which, according to the results of experiments in vitro, should set free active thrombin. He has also introduced directly into the circulating blood considerable amounts of thrombin, prepared by my method, but in neither case could metathrombin be detected in the oxalated plasma of blood drawn subsequently after 5 minutes, 30 minutes, or 1 hour. It would seem from these experiments that if metathrombin is formed in the circulating blood it must be destroyed with surprising promptness, and this conclusion was apparently supported by the observation that when metathrombin is introduced directly into the circulation it can not be detected subsequently in the plasma, that is to say, when serum containing metathrombin in demonstrable amounts is injected intravenously, the metathrombin test applied to the plasma, after bleeding and oxalating, is negative. Further experiments showed that in a mixture of serum and oxalated plasma in equal parts made outside the body no metathrombin can be detected in spite of the fact that the serum itself gives abundant evidence of metathrombin before mixture with the plasma. Either there is something in the plasma which destroys the metathrombin, and this suggestion is highly improbable, or the conditions of the mixture are such as to make it impossible to recognize the metathromhbin by the usual test, namely, the liberation of free thrombin. This latter explanation seems more probable. Plasma contains more antithrombin than serum and it is in free form so far as we know, whereas in serum the antithrombin is in combination loose or firm with thrombin. It is possible therefore that in the case of plasma or a mixture of plasma and serum metathrombin may be present and may be broken up by the alkali treatment with the liberation of some thrombin, but this latter may not be able to reveal itself owing to the presence of free antithrombin. Some evidence in favor of this explanation connected with the process of reactivation will be given in Mr. Rich's paper.[38] It will be noted that these experiments give a new aspect to the question of the existence or non-existence of metathrombin in the blood. According-

ing to the method of alkali activation no metathrombin exists in circulating blood but as appears from the foregoing facts it is evident that if small amounts of metathrombin were present in the blood its existence would not be revealed by this method. It would seem probable that thrombin is formed at times in the blood and that the presence of antithrombin constitutes a safety device to prevent any resulting formation of fibrin.

Antiprothrombin.—In some work done in my laboratory by J. McLean and published in 1916 [32] an investigation was made of the action of the various phosphatids occurring in the body in regard to their influence upon the process of coagulation. It was found in accordance with my previous results that lecithin seems to exert little or no effect and that purified cephalin has the remarkable accelerating influence described above. On the other hand two phosphatids the so-called cuorin from heart muscle prepared by the method of Erlandsen,[55] and a phosphatid prepared from the liver according to the general method of Baskoff [56] have a marked inhibitory effect upon coagulation. These latter results have been under investigation during the present year, 1916–17, and some of the results may be stated briefly here.

The substance or substances in question have been prepared from heart-muscle, liver and lymph glands. The material was obtained by extracting the dried tissue with ether. The ether extract after filtration or centrifugalization was evaporated to dryness, extracted with acetone and dissolved in a small amount of chloroform. This solution after filtration or centrifugalization was precipited at 50° C. by four times its volume of absolute alcohol. The precipitate was centrifugalized, dried, dissolved in chloroform and again precipitated at 50° C. with absolute alcohol, and this process was repeated a number of times. In the case of the extract from lymph glands two such precipitations with alcohol may suffice to free the active substance from other lipoids, but with the liver-extracts four or five or more precipitations are necessary to get rid of the accompanying cephalin. The material finally obtained was a brownish powder which when dissolved in water or salt solution gives a slightly opalescent solution that in the case of the lymph glands is nearly colorless but in the

case of the liver and the heart is more deeply colored owing possibly to some adherent pigment. Samples of the material obtained from the heart and the liver were analyzed at the Rockefeller Institute through the kindness of Dr. Levene and gave the following results:

Material from the Heart-muscle—C. 40.54, H. 6.32, N. 1.40, P. 6.69—Ash 34.86.

Material from the Liver—C. 45.06, H. 7.19, N. 5.01, P. 4.75—Ash 21.21.

In the material from the heart-muscle the ratio of N to P is 2 to 1 in accordance with Erlandsen's analysis for cuorin. On the other hand, the material from the liver gave a ratio of N to P of 1 to 2.4, a result which would indicate a relation of this substance to the jecorin described by Drechsel rather than to the heparphosphatid as isolated by Baskoff. These two substances exhibited a similar effect upon the coagulation of blood, retarding or inhibiting completely the coagulation according to the concentrations used. The material from the liver was more effective than that from the heart-muscle. It is evident that the materials obtained are different substances, one being apparently a monamino-diphosphatid and the other a diamino-monophosphatid. The great uncertainty that exists at present in regard to the structure and chemical individuality of these phosphatids, particularly as regards those obtained from the liver, is well known—but their action in regard to the process of coagulation seems to be essentially identical and it is convenient provisionally to speak of them as antiprothrombin or as antiprothrombins to indicate the nature of this action. Their solutions are capable of preventing entirely the coagulation of blood in vivo and in vitro.

Intravascular Injection.—Injection of saline solution of the antiprothrombin in the proportion of approximately 1 decigram of antiprothrombin per kilogram of animal (dog) makes the blood incoagulable, so far as spontaneous coagulation is concerned. The injections cause no general reactions in the animal, the blood pressure, heart rate and respiratory rate are unaffected, but blood withdrawn from the animal a few minutes after the injection does not coagulate. The effect of the antiprothrombin wears off grad-

ually, but the coagulation time may be greatly prolonged for hours after the injection. Observations made upon the antithrombin contents of the blood and also upon what I have called the prothrombin time (the time of clotting of the oxalated plasma when recalcified with the optimum amount of calcium) supplement these results upon the coagulation time of the whole blood and present a picture comparable to that found in the blood of hereditary hemophilics,[38] namely a great delay in the prothrombin time with indications of a distinct increase in antithrombin. In one experiment for example the following results were obtained:

1. Before injection—Prothrombin time = 5 minutes.

Antithrombin series.	Thrombin 5 drops = Clot in 5 minutes.
	Thrombin 4 drops = Clot in 10 minutes.
	Thrombin 3 drops = Floating clot in 10 minutes.
	Thrombin 2 drops = Membrane in 15 minutes.

2. Ten minutes after injection—Prothrombin time = No clot in 24 hours.

Antithrombin series	Thrombin 5 drops = Floating clot in 15 minutes.
	Thrombin 4 drops = Membrane in 30 minutes.
	Thrombin 3 drops = Membrane in 120 minutes—solid over night.
	Thrombin 2 drops = Nothing in 130 minutes—Membrane over night.

3. One hour after injection — Prothrombin time = 38 minutes.

Antithrombin series	Thrombin 5 drops = Clot in 10 minutes.
	Thrombin 4 drops = Floating clot in 15 minutes.
	Thrombin 3 drops = Membrane in 40 minutes.
	Thrombin 2 drops = Nothing in 130 minutes, but solid clot over night.

4. Two hours after injection — Prothrombin time = 12 to 14 minutes.

Antithrombin series	Thrombin 5 drops = Clot in 5 minutes.
	Thrombin 4 drops = Clot in 10 minutes.
	Thrombin 3 drops = Floating clot in 15 minutes.
	Thrombin 2 drops — Membrane in 20 minutes.

Action of the Antiprothrombin in Vitro.—Experiments made upon the action of solutions of antiprothrombin upon blood out of the body show that it prevents or retards the clotting of the blood in proportion to the concentrations used. So far no effort has been made to determine the minimal concentration

sufficient to prevent the clotting of the whole blood. Experiments made upon oxalated plasmas in regard to their clotting on re-calcification show that when the antiprothrombin is added to the oxalated plasma to give a concentration of 0.1 per cent no clotting occurs on calcifying—while with a concentration of 0.04 per cent, coagulation while still perceptibly retarded is not prevented.

The Nature of the Action of Antiprothrombin.—As indicated by the name suggested for this anticoagulant there is reason to believe that it retards or prevents coagulation by preventing the formation of thrombin and most probably by binding or combining with the prothrombin so as to prevent its activation to thrombin. This belief is based upon facts of the following kind:

1. The substance is not itself an antithrombin. It has no effect upon the reaction between thrombin and fibrinogen. When its solutions are allowed to stand in contact with thrombin for 15 minutes, as in the usual antithrombin test, and the fibrinogen solution is then added coagulation of the latter takes place as promptly as in a control in which saline (NaCl 0.9 per cent) replaces the antiprothrombin solution. In accordance with this fact it is found also that whole blood made incoagulable by this substance, either by mixture in vitro or by intravascular injection, can be made to clot upon the addition of thrombin solutions. Mere addition of water to such bloods (4 vols.) has no effect, nor the addition of cephalin solutions alone. Cephalin solutions plus thrombin are more effective than thrombin alone, a fact which falls in with my views regarding the action of the cephalin.

2. When solutions of this substance are added to whole blood the prothrombin time of the plasma, obtained by oxalating and centrifugalizing, is prolonged in proportion to the amount added. In the same way the oxalated plasma of normal blood may have its power to clot on recalcification either prolonged or prevented entirely by the addition of solutions of this material. These reactions are in accord at least with the view stated above in regard to its mode of action.

3. Solutions of prothrombin obtained from the oxalated plasma of the cat by a method previously described have their power to form thrombin completely inhibited by the presence

of this substance. This reaction was obtained repeatedly—for example:

1. Prothrombin solution 5 drops + H_2O 2 drops + $CaCl_2$ 2 drops + Fibrinogen 8 drops = Clot in 5 minutes.

2. Prothrombin solution 5 drops + Ap 2 drops + $CaCl_2$ 2 drops + Fibrinogen 8 drops = No clot in 24 hours.

Ap. in this table stands for the solution of antiprothrombin.

There seems to be no other probable explanation of the effect of this substance on prothrombbin solutions except the one offered, since the possibility of an action of the material on the other factors involved, namely, the fibrinogen or the calcium salt is easily excluded.

4. Solutions of antiprothrombin while they have no antithrombic action of their own, cause a production of antithrombin when added to the blood inside or outside the body. The effect of injections of the antiprothrombin in increasing the antithrombin content of the blood has been illustrated above. Outside the body addition of this substance to serum or to oxalated plasma causes a notable increase in its antithrombin content. This reaction is constant and unmistakable. It may be illustrated by a single example. The experiment in this case was made with the clear plasma obtained by centrifugalizing oxalated cat's blood. The plasma was heated to 54° C. to precipitate fibrinogen. Two mixtures were then made—A the oxalated plasma plus an equal volume of water, and B the oxalated plasma plus an equal volume of a solution of antiprothrombin. The antithrombin content of these mixtures was determined in comparison with a third mixture, C composed of equal volumes of water and the solution of antiprothrombin.

I. Thrombin 5 drops + A 1 drop — incubation 15 minutes — Fibrinogen 10 drops = clot in 5 minutes.

Thrombin 4 drops — A 1 drop — incubation 15 minutes — Fibrinogen 10 drops = clot in 10 minutes.

Thrombin 3 drops — A 1 drop —incubation 15 minutes — Fibrinogen 10 drops — clot in 10 minutes.

Thrombin 2 drops — A 1 drop — incubation 15 minutes — Fibrinogen 10 drops — clot in 15 minutes.

II. Thrombin 5 drops + B 1 drop — incubation 15 minutes — Fibrinogen 10 drops — no clot in 2½ hours.

Thrombin 4 drops + B 1 drop — incubation 15 minutes — Fibrinogen 10 drops — no clot in 2½ hours.

Thrombin 3 drops + B 1 drop — incubation 15 minutes — Fibrinogen 10 drops — no clot in 2½ hours.

Thrombin 2 drops + B 1 drop — incubation 15 minutes — Fibrinogen 10 drops — no clot in 2½ hours.

III. Thrombin 5 drops + C 1 drop — incubation 15 minutes—Fibrinogen 10 drops — clot in 5 minutes.

Thrombin 4 drops + C 1 drop — incubation 15 minutes—Fibrinogen 10 drops — clot in 5 minutes.

Thrombin 3 drops + C 1 drop — incubation 15 minutes — Fibrinogen 10 drops — clot in 5 minutes.

Thrombin 2 drops + C 1 drop — incubation 15 minutes — Fibrinogen 10 drops — clot in 5 minutes.

Similar results were obtained when mixtures were made of blood serum and solutions of antiprothrombin. In all cases the antithrombic action of the serum was greatly increased. It is difficult or impossible to give a satisfactory explanation of this reaction. Evidently there is some substance in the plasma and the serum with which the antiprothrombin reacts so as to intensify or augment the antithrombic action of the blood. We might suppose that the antiprothrombin is converted in some way to antithrombin, or that it splits off antithrombin from a mother substance, or that it serves simply to sensitize or intensify the action of the antithrombin already present. Experiments have shown that the material in the plasma or serum with which the antiprothrombin reacts is destroyed by heating at 70° C., or between 65° and 70° C., and this fact would indicate that it is either the metathrombin or the antithrombin that is concerned. If the metathrombin is a compound of thrombin and antithrombin it is scarcely probable that the antiprothrombin acts upon it in such manner as to split off the antithrombin, since as has been stated the antiprothrombin appears to have no action whatever on purified thrombin. Moreover, the reaction is exhibited as markedly by plasma as by serum, and we have no warrant at present for assuming the existence of considerable amounts of

metathrombin in plasma. By exclusion, therefore, we are forced to assume as a possibility that the antiprothrombin simply intensifies the activity of the antithrombin in the blood. Whether or not this conclusion is justified by further experiments there is no doubt in regard to the main fact upon which emphasis is laid at present, namely, that there are two substances in the body which are capable of retarding the process of clotting. One of these, antithrombin, is present in circulating blood and acts by preventing the reaction between thrombin and fibrinogen, the other, antiprothrombin, may or may not be present in the circulating blood, but it is present in some form in certain tissues and it acts by preventing the conversion of prothrombin to thrombin. In the literature of coagulation this distinction has not been made. As a rule authors have designated as antithrombin any substance, other than precipitants of calcium or fibrinogen, that prevents the clotting of blood. Schmidt, it is true, recognized clearly enough that inhibiting substances may act by retarding the formation of thrombin and in fact this was his suggestion in regard to the two protein materials, cytoglobin and preglobulin which he extracted from various tissues. In the later literature Doyon especially has attempted to prepare antithrombin by several different methods and to determine its chemical nature. His final conclusion [44] is that it is essentially a nucleic acid. If we accept the definition of antithrombin given above it would appear that Doyon is wrong in this conclusion. Thymus nucleic acid (sodium salt) does have a moderate inhibitory effect upon coagulation, but it has no influence at all of this character on the reaction between thrombin and fibrinogen, so that it cannot be identified with antithrombin. Nor is it probable that its action is identical with that of antiprothrombin, since its addition to plasma causes no increases in antithrombic power. Schickele,[42] in a paper already referred to, states that the pressure-juice of several tissues, particularly of the uterine mucous membrane, shows the presence of an antithrombin. His experiments have been repeated in my laboratory by Dr. Jessie L. King. In accordance with his results it has been found that the pressure-juice from the uterine mucous membrane does con-

tain a material capable of retarding the clotting of blood, but analysis of its action shows that this substance belongs to the group of antiprothrombins rather than to the antithrombins—that is to say, the juice obtained from the washed membrane with a Buchner's press has no retarding influence at all upon the reaction between thrombin and fibrinogen but it causes a prolongation of the prothrombin time, it retards or prevents the reaction between prothrombin and calcium, and when added to plasma or serum causes in it a marked increase in antithrombic power.

This result, together with those described above, indicates that antiprothrombin has a widespread occurrence in the body and constitutes in all probability the inhibitory group or complex which Schmidt was led to believe exists in many tissues conjugated with protein to form the substance designated by him as cytoglobin. Conradi [57] has put on record the fact that many tissues when submitted to autolysis develop a substance which has a retarding influence upon the coagulation of blood. He speaks of this substance under the general name of antithrombin, but leaves the question open as to whether it acts on thrombin, prothrombin or zymoplastic substance. I have not repeated Conradi's experiments but the facts stated above make it very probable that the material he describes will turn out to be this widely distributed antiprothrombin.

The Relation Between Cephalin, Antithrombin and Antiprothrombin.—That cephalin is able to neutralize or antagonize antithrombin as it occurs in blood-plasma has been shown by experiments, such as are quoted in the preceding pages. Similar experiments indicate clearly that there is a distinct antagonism between cephalin and the antiprothrombin as regards the process of clotting. When blood is prevented from clotting by the addition of an excess of antiprothrombin the further addition of cephalin may not be adequate to induce clotting, but when the amount of antiprothrombin used is not in excess but is sufficient simply to delay greatly the process of clotting then cephalin antagonizes its action and brings on prompt clotting. This result was obtained in experiments made with a prothrombin

extract, prepared by the method previously described, a solution of antiprothrombin prepared from the liver and used in a concentration of 0.04 per cent, a solution of fibrinogen, a solution of freshly made cephalin and a solution of calcium chloride 0.5 per cent. Mixtures were made according to the following schema in which Ap stands for antiprothrombin:

Five drops of prothrombin extract plus 4 drops of Ap, plus 3 drops of water, plus 2 drops of calcium chloride, plus 10 drops of fibrinogen gave an imperfect floating clot in 40 to 45 minutes.

Five drops of prothrombin extract plus 4 drops of Ap, allowed to stand for 40 minutes, then added 3 drops of cephalin solution, 2 drops of calcium chloride and 10 drops of fibrinogen. The mixture gave a solid clot in 4 to 5 minutes. A control mixture of prothrombin extract, calcium chloride and fibrinogen without the addition of antiprothrombin clotted in 5 to 10 minutes.

This experiment was repeated in various forms with similar results. It would appear, therefore, that the combination formed between prothrombin and antiprothrombin can be broken up in the presence of cephalin. While this reaction does not demonstrate that in the normal circulating blood prothrombin is covered or protected by an antiprothrombin it is in accord with such a view. The activation of prothrombin by calcium is readily inhibited by the antiprothrombin and the effect of the latter in turn is antagonized by cephalin. It would seem highly probable that these factors enter into the coagulation process in the way described, but further experiments and a better knowledge of the chemical properties of the substances concerned are necessary before a definite statement can be made in regard to the precise way in which the equilibrium of the circulating blood is altered by the addition of cephalin.

One of the interesting results of the experiments made with antiprothrombin is the suggestion they offer of a possible explanation of the condition of hemophilia. Blood to which antiprothrombin has been added in amounts sufficient to cause a marked slowing of the time of coagulation presents a picture which is identical with that exhibited by hemophilic bloods. There is the

same prolongation of the time of spontaneous coagulation, the same retardation in the prothrombin time and the same tendency toward an increase in antithrombic action. The suggestion that the condition of hemophilia may be due to abnormal amounts of antiprothrombin in the blood is one that can be submitted to experimental examination as soon as an adequate method is devised for determining the presence and amount of antiprothrombin in circulating blood.

In conclusion attention may be called briefly to the fact that we have now some knowledge concerning two substances, both belonging to the group of phosphatids which influence the clotting of blood in opposite ways, one, cephalin, causing an acceleration, the other, antiprothrombin, a retardation. Neither of these substances apparently provokes an injurious reaction in the living animal, and we may hope therefore that they will find a suitable application in experimental work and possibly in the therapeutic treatment of disorders of coagulation.

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